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Mary J. Daniel

ISOLATION AND IDENTIFICATION OF OBLIGATELY ANAEROBIC BACTERIA FROM BOVINE LUNG ABSCESSES

BY

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This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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INTRODUCTION

For many years, the role of anaerobic bacteria in human disease was considered important only in such clostridial diseases as gas gangrene, botulism and tetanus. In the last 10 years, however, the development of new methods to isolate and identify anaerobic bacteria has established the pathogenic role of non-sporeforming anaerobic bacteria in a wide variety of clinical infections in humans. Bacteroides fragilis is now known to account for approximately one-fourth of all anaerobic bacteria isolated from human clinical specimens (27).

Recent advances in anaerobic bacteriology likewise have established that anaerobic bacteria occur throughout the human body as indigenous flora. Anaerobic bacteria are found as normal flora on the skin and all mucous membrane surfaces. Since almost all anaerobic bacterial infections originate endogenously, it is important to know the specific types of anaerobic bacteria present as normal flora at various sites on and in the human body. This enables one to predetermine which anaerobic bacteria may be involved in a particular infection and whether or not a particular isolate is significant. For example, the normal oral cavity of humans is mainly composed of Fusobacterium nucleatum, Bacteroides melaninogenicus, streptococci, peptococci, peptostreptococci, lactobacilli, eubacteria and bifidobacteria (65). Under certain conditions predisposing to aspiration, anaerobic bacteria from the oral cavity can be carried into the lung. If the proper conditions exist in the lung (tissue necrosis from trauma, surgery, aerobic and facultative bacterial

infection), anaerobic bacteria can establish an infection such as a lung abscess. In human lung abscesses, the most frequently encountered anaerobic bacteria are F. nucleatum, B. melaninogenicus, peptostreptococci, peptococci and eubacteria (30). The oral cavity is therefore the major source of anaerobic pathogens involved in lung abscesses.

With the exception of clostridia, little information is known about the normal flora of animals or the identity of anaerobic bacteria involved in nonspecific infections in animals. In contrast to human medicine, nonspecific infections such as anaerobic pleuropulmonary infections have been relatively ignored in veterinary medicine. Thus, the purpose of this research is to determine the relative incidence and species of anaerobic bacteria present in bovine lung abscesses and to speculate on what role normal flora play in this type of infection.

Since anaerobic bacteria have not been isolated to any great degree from animal infections, the susceptibility patterns of anaerobic bacteria isolated from human infections have been used by the veterinarian as a guide for antibiotic therapy in the treatment of anaerobic infections in animals (40). This practice is questionable because the antibiotic susceptibility of anaerobic bacteria isolated from animals may not be identical to that demonstrated in man. Therefore, the second objective of this study is to provide information on the antibiotic susceptibility of anaerobic bacteria isolated from bovine lung abscesses.

For purposes of simplicity, obligately anaerobic bacteria, unless stated as such, will be referred to in this text as anaerobic bacteria or anaerobes.

LITERATURE REVIEW

Presence of Anaerobic Bacteria in Animal Infections

The role of anaerobic bacteria in clinical infections in humans has been widely studied (3, 27, 65, 76, 86). Information concerning anaerobic bacteria involved in animal infections is scarce.

Biberstein et al. (9) concentrated on isolating Bacteroides melaninogenicus from various animal diseases. B. melaninogenicus was isolated 102 times from 2,164 samples (4.7%) with the highest relative incidence in cats and lowest in horses. In a single sample, B. melaninogenicus was usually associated along with one to five additional bacterial species.

In a one-year study of animal diseases Berkhoff and Redenbarger (6) recovered anaerobic bacteria from 61.6% of the specimens. In approximately 22% of the specimens anaerobic bacteria were the only organisms isolated. Clostridium species, Bacteroides species, Fusobacterium species and anaerobic cocci represented 50.0%, 12.8%, 6.4% and 1.2% of the anaerobic bacteria isolated, respectively. Clostridium perfringens was the most common anaerobic species isolated. Anaerobic bacteria had the highest frequency of isolation from dogs and the lowest from cats. Specimens which yielded the greatest number of anaerobic bacteria included thoracic fluid, muscle, liver, abscesses and lung. In a further study Berkhoff (7) examined 300 specimens and reported that 56.3% yielded anaerobic bacteria.

Obligately and facultatively anaerobic bacteria were present together in 37.2% of the specimens. Clostridium species, Bacteroides species, Fusobacterium species, Actinomyces species and Propionibacterium species represented 46.0%, 15.1%, 14.3%, 11.1% and 5.6% of the 126 anaerobic bacteria isolated, respectively. In specimens collected from cattle, Fusobacterium necrophorum was isolated from a lung abscess, liver abscess and trachea. F. necrophorum is recognized as an animal pathogen and is known to cause numerous diseases in animals (45).

Results of a study by Hirsh et al. (38) completely disagreed with previous reports (6, 7). Anaerobic bacteria were recovered from only 26% of the bacteriologically positive specimens. Of the 641 anaerobic isolates, Bacteroides species, Peptostreptococcus species and Fusobacterium species composed 46%, 15% and 6%, respectively. Clostridium species represented only 8% of the total isolates. Bacteroides melaninogenicus and Peptostreptococcus anaerobius were the two most commonly isolated anaerobic species. Similar results were reported by Prescott (56). Of the 205 isolates, Bacteroides melaninogenicus, B. oralis, Fusobacterium necrophorum and Peptococcus indolicus were the four most commonly isolated anaerobic bacteria. Bovine specimens yielded the greatest number of anaerobic bacteria including all of the P. indolicus isolates.

Berg et al. (5) studied the occurrence of anaerobic bacteria isolated from dog and cat infections. Anaerobic bacteria were recovered from 37% of the 304 specimens evaluated. Eighteen percent of the specimens contained only anaerobic bacteria, whereas aerobic

and anaerobic bacteria were recovered in mixed cultures from 24% of the specimens. Clostridium species, anaerobic cocci, Bacteroides species and Fusobacterium species represented 30.5%, 11.8%, 31.0%, and 10.6% of the 111 anaerobic bacteria isolated, respectively. The four most common species isolated were Clostridium perfringens, Bacteroides melaninogenicus, Peptostreptococcus anaerobius and Fusobacterium necrophorum. Samples yielding the highest frequency of anaerobic bacteria included abscesses, draining tracts, granulomas, deep wounds, bone diseases and abdominal lesions. Recovery of anaerobic bacteria from respiratory tract specimens was very poor. Love et al. (49) sampled 36 subcutaneous abscesses in cats and reported that 72% of the 168 isolates were anaerobic bacteria. Bacteroides species, Fusobacterium species, Peptostreptococcus species and Clostridium species represented 28.6%, 19.0%, 10.7%, 6.5% of all isolates, respectively. The most common anaerobic species recovered was Peptostreptococcus anaerobius.

Oxygen Sensitivity of Anaerobic Bacteria

Traditionally, anaerobic bacteria have been defined as bacteria which can grow only in the absence of oxygen. Prevention of growth by oxygen can be attributed to the inactivation of intracellular key components or to the production of toxic substances such as oxygen radicals and hydrogen peroxide intracellularly or extracellulary. Besides oxygen radicals and hydrogen peroxide, adverse oxidation-reduction potentials of the medium can also be

involved in affecting the growth of anaerobic bacteria. These factors contribute to the various degrees of sensitivity to oxygen exhibited by anaerobic bacteria.

Loesche (48) determined the oxygen sensitivity of various anaerobic bacteria. Agar plates were streaked in an anaerobic glove box and then placed in anaerobic jars containing 10% hydrogen, 10% carbon dioxide, 68 to 80% nitrogen, and varied amounts of air to give oxygen concentrations ranging from 0 to 12%. Based on his results, anaerobic bacteria were classified into two major categories. Strict anaerobes did not exhibit agar surface growth at oxygen tension greater than 0.5%. Anaerobic bacteria included in this group were Treponema macrodentium, T. denticola, T. oralis, Clostridium haemolyticum, Selenomonas ruminatium, Butyrivibrio fibrisolvens, Succinivibrio dextrinosolvens and Lachnospira multiparus. Moderate anaerobes were capable of growth at oxygen levels as high as 2 to 8%. Species assigned to this group were Bacteroides fragilis, B. melaninogenicus, B. oralis, Fusobacterium nucleatum, Clostridium novyi type A and Peptostreptococcus elsdenii.

Fredette et al. (28) measured the sensitivity of eight species of anaerobic bacteria to air and to pure oxygen at fifteen and thirty pounds pressure while multiplying in agar deeps. The zone of inhibition downward from the surface of the agar deep increased in size as the oxygen pressure was increased. Clostridium perfringens being the most aerotolerant of the organisms examined had the smallest zone of inhibition. Bacteroides fragilis had the largest zone of

inhibition and therefore is the most sensitive to the effects of oxygen. They concluded the zone of inhibition is proportional both to the logarithm of the oxygen pressure and to the logarithm of the dilution of the culture.

The exact mechanisms of oxygen toxicity have not been clearly established. Many hypotheses have been stated trying to explain oxygen toxicity.

Morris (52) suggests the presence of free oxygen in a culture medium is incompatible with achieving and maintaining a low culture E_h necessary for proper growth of anaerobic bacteria. He concluded a low E_h value, established by the use of reducing agents, is essential for growth initiation especially from small inoculum. Recently however questions have been raised as to whether oxygen exerts its toxic effects as an excellent oxidant or as a toxic agent itself. O'Brien and Morris (53) studied the effects of E_h on the growth and metabolism of Clostridium acetobutylicum. Addition of potassium ferricyanide increased the E_h of the anaerobic culture to +370 mv. The chemical increase did not affect normal rates of growth, glucose consumption or the production of acetate, butyrate, and pyruvate. Aeration of the high E_h culture immediately halted growth. Addition of dithiothreitol to the oxygenated culture did not permit growth to occur even though the E_h was lowered to -50 mv. Walden and Hentges (79) demonstrated the effects of E_h on the growth of Peptococcus magnus, Clostridium perfringens and Bacteroides fragilis. The E_h of the anaerobic culture medium was raised by either aeration

with pure oxygen or addition of 10% potassium ferricyanide. The multiplication of all three bacteria was inhibited by the presence of oxygen in both the negative and positive ranges of oxidation-reduction potential. In the absence of oxygen, multiplication of all three bacteria occurred at either negative or positive oxidation-reduction potentials. These investigators concluded the oxygen inhibited growth by some action other than increasing the E_h potential.

Working with Clostridium acetobutylicum, O'Brien and Morris (53) concluded that since oxygen is an avid electron acceptor, growth of the organism is halted by its inability to maintain intracellular concentrations of specific electron donors such as NADH and NADPH. The unrewarding task of detoxifying oxygen drains the organism of reducing power which is necessary for biosynthetic purposes and growth.

Oxygen toxicity may be attributed to products formed by the interaction of oxygen with components of the culture media and/or the organisms (52). Toxic products such as organic peroxides, aldehydes and free radicals can accumulate in complex culture media exposed to oxygen. Toxic products are also formed by the reaction of oxygen with reduced cell components such as tetrahydropteridines and flavoproteins and by certain oxidases and flavin dehydrogenases. The toxic products formed are hydrogen peroxide, superoxide anion, hydroxyl radical and singlet oxygen. Carlsson et al. (17) demonstrated the formation of hydrogen peroxide and superoxide

radical in an anaerobic medium exposed to atmospheric oxygen. They concluded that the formation of toxic products was directly related to the presence of glucose and phosphate in the culture medium. By autoclaving a culture medium containing glucose and phosphate under alkaline conditions, the rate of hydrogen peroxide and superoxide radical formation was greatly enhanced upon exposure to atmospheric oxygen. The autoxidation of cysteine was studied by Carlsson et al. (18). Upon exposure to atmospheric oxygen, cysteine will in the presence of transitional metal ions autoxidize to cystine resulting in the formation of hydrogen peroxide. The combination of hydrogen peroxide and metal-ion catalyst was found to be very toxic to Peptostreptococcus anaerobius. Catalase, peroxidase and metal-ion chelating agents afforded complete protection to this organism.

Protection of anaerobic bacteria against hydrogen peroxide formed intracellularly and extracellularly upon exposure to oxygen is provided by the enzyme catalase. Many investigators have demonstrated catalase activity in Bacteroides fragilis, B. distasonis, B. thetaiotaomicron and Propionibacterium acnes. The production of catalase by these bacteria has been shown to be directly related to several important factors. Gregory et al. (31) studied catalase synthesis in Bacteroides fragilis and found that glucose, lactose, fructose, raffinose, starch, mannose, sucrose, galactose and maltose at concentrations of 0.5% wt./vol. repressed catalase synthesis. B. fragilis grown in the absence of these carbohydrates produced 25 to 30 units of catalase per milligram of protein. Gregory et al.

(32) reported catalase activity in Bacteroides distasonis responded to the heme levels in the growth medium. The presence of hemin in the culture medium induced catalase synthesis. Besides hemin, vitamin K_1 was added to the culture medium and even though it alone had no effect on growth or catalase activity it was synergistic with hemin in elevating the catalase activity. Wilkins et al. (85) tested catalase production in several species of Bacteroides and concluded that catalase activity depended not on hemin concentration but upon the type of medium used and the manner in which hemin was added to the medium. Catalase production was best in a broth medium which had hemin added after sterilization. They concluded sterilization of hemin must affect its availability in the culture medium.

Superoxide dismutase is classified as a metalloflavoenzyme. According to Fridovich (29) procaryotes possess two distinct types of superoxide dismutases, the iron-containing superoxide dismutase found in the periplasmic space and the manganese-containing superoxide dismutase found in the cytoplasm. The procaryotic cell may contain both, one, or neither of these enzymes. By the use of the trivalent and divalent states of the metals, the principle function of the superoxide dismutases is to catalyze the dismutation of superoxide anions to yield hydrogen peroxide and triplet oxygen.

In 1971 the superoxide dismutase theory of obligate anaerobiosis was conceived by McCord et al. (51). The theory stated that oxygen sensitivity exhibited by anaerobic bacteria is due to their complete lack of superoxide dismutase. They suggested that

since anaerobic bacteria lack a cytochrome system needed for respiration and therefore do not use oxygen as a terminal electron acceptor, superoxide anions are not generated and superoxide dismutase is not required for cell protection. This statement was supported by the fact that no detectable superoxide dismutase activity was found in Veillonella alcalescens, Clostridium pasteurianum and C. acetobutylicum.

In 1975 detectable levels of superoxide dismutase were reported in Clostridium acetobutylicum, C. bifermentans, C. butyricum, C. pasteurianum, C. perfringens and C. sporogenes by Hewitt and Morris (36). Carlsson et al. (16) demonstrated superoxide dismutase activity in Bacteroides fragilis, B. thetaiotaomicron, B. ovatus, B. vulgatus and B. distasonis. Since all strains survived for one week on the surface of agar exposed to air, atmospheric oxygen is considered bacteriostatic rather than bactericidal.

The fecal isolates, Peptostreptococcus anaerobius, Clostridium aminovalericum, Fusobacterium nucleatum and Bacteroides melaninogenicus, were classified as intolerant anaerobes by Rolfe et al. (59). These intolerant anaerobes survived less than two hours upon oxygen exposure and none possessed superoxide dismutase. The moderately tolerant anaerobes, Bacteroides fragilis, B. vulgatus and Propionibacterium acnes, survived from 48 to more than 72 hours upon exposure to oxygen and all demonstrated superoxide dismutase activity. The oxygen tolerance exhibited by certain anaerobic

bacteria can be related partly to the protective enzymes such as catalase, peroxidase and superoxide dismutase produced by the cell.

Inducing the production of superoxide dismutase in Bacteroides fragilis was studied by Privalle and Gregory (57). Exposing midlog cultures to two percent oxygen for two hours increased the superoxide dismutase activity three to five fold as compared with midlog cultures held under anaerobiosis for the same time.

Many investigators have suggested that a pre-requisite to virulence in an anaerobe is its capacity to tolerate small concentrations of oxygen. Superoxide dismutase could therefore be described as a virulence factor which allows certain pathogenic anaerobic bacteria to survive in well oxygenated tissues until a low oxidation-reduction potential is reached. Tally et al. (75) support these theories for two reasons. Anaerobic bacteria classified as extremely oxygen sensitive do not possess superoxide dismutase and are not usually associated with infections whereas aerotolerant and intermediate anaerobes are involved in infections. The majority of the aerotolerant and intermediate anaerobes do possess superoxide dismutase to a certain extent and this could account for the pathogenicity exhibited by these types of anaerobes.

Anaerobic Transport Systems

Proper collection and rapid transport of clinical specimens to the laboratory are two fundamental techniques necessary for the maximum recovery of anaerobic bacteria. Many pathogenic anaerobic bacteria are not aerotolerant; therefore transport systems and

transport media have been constructed to allow adequate protection of the specimen during transit.

"An ideal transport system should be nonselective as well as nonsupportive, capable of maintaining viability without promoting growth or appreciably altering the relative proportion of various organisms initially present in the clinical specimen," Chow et al. (19).

The use of swabs for collecting and transporting clinical specimens is considered to be less efficient than any other method and should be used only when purulent material is limited at the infection site. Collee et al. (21) demonstrated that anaerobic bacteria collected by a non-toxic swab die in transit due to dessication rather than actual oxygen inactivation. They also found that loss of recovery of anaerobic bacteria during transit can be attributed to the retention of bacteria on the swab.

Various types of transport media have been devised to prevent dessication of swabs during transport. Christian and Ederer (20) compared trypticase soy broth agar, Stuart transport medium with charcoal, Amies transport medium and modified Stuart transport medium to determine their adequacy as a transport medium. Each transport medium maintained the viability of Bacteroides fragilis, Peptostreptococcus anaerobius and Clostridium perfringens for up to 24 hours.

Barry et al. (4) compared the survival of anaerobic bacteria on swabs stored in Amies transport medium without charcoal and in

dry test tubes. Bacteroides fragilis, B. melaninogenicus, Peptostreptococcus sp. and Clostridium sordelli all survived better in the transport medium; however viability decreased over a short period of time. Yrios et al. (87) contrasted the growth rates of Bacteroides thetaiotaomicron, B. asaccharolyticus and Fusobacterium nucleatum inoculated onto swabs and placed into dry gassed-out tubes, dry aerobic tubes and tubes containing a modified Stuart transport medium. They found the viability of all three bacteria was maintained for at least two hours in the modified Stuart medium and the dry gassed-out tubes.

Ederer and Christian (24) evaluated seven different types of swab transport systems with each having a different transport medium incorporated into the system. They found no system to be the best for transporting specimens and that specimens should be cultured within four hours for adequate recovery.

Holdeman and Moore (39) suggest that a two-tube system be used for collecting specimens on swabs when oxygen-free CO_2 is not available at the time the specimen is taken.

Wilkins and Jimenez-Ulate (83) have developed a new apparatus for transporting swabs, fluid and tissue specimens. The anaerobic specimen transport device is constructed to limit the amount of oxygen entering the sample to a maximum of 2%. The anaerobic specimen transport device maintained the viability of Bacteroides melaninogenicus, Fusobacterium necrophorum, F. mortiferum,

Peptostreptococcus anaerobius, Clostridium innocuum, C. perfringens, C. ramosum and Peptococcus magnus for up to 48 hours. Hill (37) evaluated the efficiency of the Anaerobic Specimen Collector (Becton, Dickinson and Co., Cockeysville, Md.) in maintaining the viability of bacteria in known polymicrobial mixtures. Mixture #1 contained Bacteroides fragilis, Peptostreptococcus anaerobius and Escherichia coli. The Anaerobic Specimen Collector held the three species in equal proportions for 72 hours. Mixture #2 consisted of B. fragilis, P. anaerobius, Fusobacterium nucleatum, Staphylococcus epidermidis and Pseudomonas aeruginosa. F. nucleatum decreased 1 log after 72 hours however the other members of mixture #2 remained in relatively equal proportions for 72 hours.

Finegold et al. (26) considered aspiration with needle and syringe to be the best method for collecting specimens. The aspirated specimen can be injected into a bottle or tube containing oxygen-free CO_2 and a few drops of resazurin indicator and transported to the laboratory. They also suggest that the syringe and needle assembly containing the specimen can be directly transported to the laboratory if the needle is inserted into a sterile rubber stopper. Chow et al. (19) studied the survival of anaerobic bacteria in a commercially available gassed transport system referred to as Anaport (Scott Laboratories, Fiskeville, R.I.). Bacteroides fragilis, Peptostreptococcus intermedius, Veillonella parvula, Clostridium perfringens and Propionibacterium acnes all survived for 72 hours in the Anaport vials. Eubacterium lentum was the only

anaerobe which could not be recovered after 8 hours in the transport vial.

Helstad et al. (35) compared the recovery rates of anaerobic bacteria in three anaerobic transport systems. The transport systems consisted of specimen fluid injected into a gassed-out tube, a swab placed in Cary and Blair medium and a swab placed in a gassed-out tube containing 2 to 3 drops salts solution. After 48 hours, 97% of the anaerobic bacteria could be recovered from the fluid injected into a gassed-out tube while the swab in the transport medium and the swab in the gassed-out tube recovered 92% and 85% of the anaerobic bacteria, respectively. Hallander et al. (33) compared transportation of clinical specimens in a stoppered syringe, anaerobic transporter, a swab placed in a pre-reduced, anaerobically sterilized (PRAS) transport medium and a swab placed on an aerobic agar slant. The stoppered syringe and anaerobic transporter allowed the best viability of Fusobacterium nucleatum, Bacteroides pneumosintes and Veillonella alcalescens up to 62 hours. McConville et al. (50) evaluated three commercially available transport systems; Anaerobic Specimen Collector (Becton, Dickinson and Co.); Anaswab (Scott Laboratories); and Trans-Cul with charcoal (Wampole, Cranbury, N.J.) for the recovery of anaerobic bacteria from wounds. They concluded all three systems were equally effective in recovering anaerobic bacteria regardless of the amount of specimen cultured and the length of time from collection until initial plating.

Attebery and Finegold (2) developed a miniature anaerobic jar for tissue transport. This "mini-jar method" uses a modified copper sulfate-steel-wool system to achieve anaerobiosis. Fusobacterium fusiforme and Bacteroides melaninogenicus injected separately into a small portion of rat liver survived in the mini-jar for 5 days and 24 hours, respectively.

Phenethyl Alcohol as a Selective Agent

Lilley and Brewer (47) reported that phenethyl alcohol at a concentration of 0.25% was suitable as a selective agent for gram-positive organisms. Further work by Berrah and Konetzka (8) verified the selectivity of phenethyl alcohol. All gram-negative bacteria except Pseudomonas fluorescens were inhibited at a concentration of 0.25%. Mycobacterium phlei and M. smegmatis were the only gram-positive organisms inhibited at this specific concentration. Results indicated that phenethyl alcohol served as a bacteriostatic agent against gram-negative bacteria by its ability to inhibit selectively and reversibly DNA synthesis. Protein synthesis was not affected by phenethyl alcohol. Dowell et al. (22) studied the effects of phenethyl alcohol on the growth of various anaerobic bacteria. All strains of Bacteroides, Fusobacterium, Sphaerophorus, Peptostreptococcus and Peptococcus grew well in thioglycolate broth containing 0.25% phenethyl alcohol.

Antibiotic Susceptibility Testing of Anaerobic Bacteria

In recent years considerable research has been done on the antibiotic susceptibility testing of anaerobic bacteria isolated from human infections. Many laboratories have reported conflicting results due to the absence of a standardized method. Within the last eight years various techniques and methods have been developed in an effort to standardize the procedure for defining the susceptibility of anaerobic bacteria to antibiotics.

The standardization of antibiotic susceptibility testing is dependent upon numerous factors known to influence the activity of certain antibiotics. These factors include the type of bacteriological medium used, the pH of the medium, the ion content of the medium, the addition of blood products, the number of organisms in the inoculum, the incubation temperature and the concentration of carbon dioxide (78). Rosenblatt and Schoenknecht (60) studied the relationship between CO_2 concentration in the atmosphere and the pH change in Mueller-Hinton agar. A concentration of 4-5% CO_2 in the incubation atmosphere decreased the pH of the medium over a 25 hour period. Gentamicin, streptomycin and kanamycin were less active in the acidic medium. Chloramphenicol and erythromycin were apparently more active in an alkaline medium whereas tetracycline was most effective in an acidic environment. Ampicillin and cephalothin were apparently not affected by the changes in pH. Rahimi et al. (58), using two inoculum sizes, 10^4 cells/ml and 10^5 cells/ml, and three different agar bases, tested the activity

of four antibiotics against Bacteroides fragilis and Peptococcus species. Agar bases tested were Mueller-Hinton, brucella and brain heart infusion. Penicillin-G and clindamycin showed no significant differences in minimal inhibitory concentrations when tested against different inoculum sizes and media. The minimal inhibitory concentrations of tetracycline and chloramphenicol were generally affected by both inoculum size and the types of media used.

In 1972 Sutter et al. (70) proposed a standardized disc diffusion susceptibility test for anaerobic bacteria. Testing various strains of anaerobic bacteria, they determined thioglycolate broth to be the best medium for growing the inoculum. Brucella agar supplemented with sheep blood and menadione was chosen for the disc diffusion test. By using additional standard techniques such as 5 µg discs of tetracycline, the GasPak system and overnight incubation, the 100 strains of Bacteroides fragilis which were tested gave better correlation between minimal inhibitory concentrations and zone diameter data. When this procedure was applied to 63 strains of B. fragilis isolated between 1970 and 1972, they found 24 (38.1%) of the strains to be susceptible to tetracycline.

Bodner et al. (12) using a modified Kirby-Bauer procedure tested 70 strains of Bacteroides fragilis for susceptibility to nine antibiotics. The majority of the strains were resistant to erythromycin, tetracycline, penicillin, vancomycin and cephalothin. Clindamycin, lincomycin, chloramphenicol and carbenicillin were effective against 90% of the strains.

In a disc diffusion method proposed by Wilkins et al. (81), the pour-plate method was used instead of swabs to inoculate the plates. Air exposure, method of anaerobiosis, incubation time, method of media perparation and inoculum size had no significant effect on inhibition zone diameters. Good correlation was achieved between the zone diameters and the minimal inhibitory concentrations determined by the broth dilution method. Forty percent of the 50 strains of Bacteroides fragilis tested were resistant to tetracycline. This diffusion method, however, does have some disadvantages; organisms which fail to produce confluent growth should not be used, fast-growing anaerobes such as Clostridium perfringens cannot be used and slow-growing anaerobes are not suited for this method (10).

The standardized disc diffusion method developed by Sutter et al. (70) was used by Sapico et al. (64) to determine the antibiotic susceptibility of Clostridium perfringens. All of the 43 strains tested were susceptible to penicillin-G, doxycycline, minocycline, vancomycin, chloramphenicol and clindamycin. Ninety percent of the strains were resistant to erythromycin while 58.1% and 62.8% were susceptible to lincomycin and tetracycline, respectively.

Using the standardized disc diffusion method developed in 1972, Sutter and associates (71) tested the susceptibility of 100 strains of Bacteroides fragilis to six antibiotics. Clindamycin and chloramphenicol were the most effective antibiotics. The majority of the strains were resistant to penicillin-G, vancomycin,

lincomycin and erythromycin. The inhibition zone diameters exhibited by clindamycin, chloramphenicol, lincomycin, penicillin-G and vancomycin correlated well with minimal inhibitory concentrations determined by the agar dilution method.

Kwok et al. (43) developed a disc diffusion method to determine the antibiotic susceptibility of slow-growing anaerobes. Fifty-five strains including the genera Peptococcus, Peptostreptococcus, Megasphaera, Veillonella, Eubacterium, Bifidobacterium, Clostridium and Fusobacterium were tested against eight antibiotics. One hundred percent of the strains were susceptible to chloramphenicol. Clindamycin, penicillin-G and vancomycin were fairly active against most of the strains. The majority of the strains, however, varied in their susceptibility to lincomycin, tetracycline, doxycycline and minocycline. Since correlation between inhibition zone diameters and minimal inhibitory concentrations was good, the zone diameter values could be used to predict the susceptibility of slow-growing anaerobes.

A modified broth-disk method has been developed by Wilkins and Thiel (82). In this method the antibiotic disks are added anaerobically to pre-reduced brain heart infusion broth to simulate the concentration achievable in the blood. The results of the modified broth-disk method and broth dilution method were compared for each antibiotic. There was 100% correlation between the modified broth-disk method and broth dilution method with chloramphenicol and cephalothin. The correlation exhibited by

penicillin-G, ampicillin, clindamycin, tetracycline and erythromycin was 99%, 98%, 99%, 96% and 84%, respectively. Blazevic (11), using 110 strains of anaerobic bacteria, compared the results of the modified broth-disk method with agar dilution technique. Correlation between the two methods was 100% for clindamycin. The correlation seen for ampicillin, cephalothin, chloramphenicol, erythromycin, penicillin-G and tetracycline was 97%, 96%, 99%, 90%, 93% and 92%, respectively. The overall agreement between the two methods was 95.6%.

The modified broth-disk method has many advantages (84). Advantages include media which are commerically available and the fact that most anaerobic bacteria can be tested by this system. This method is also quick, accurate, reliable, easy to interpret and simple to use.

The modified broth-disk method has been made even simpler by Kurzynski et al. (42). Alternations in the modified broth-disk procedure include replacing the pre-reduced brain heart infusion broth with thioglycolate broth, the use of aerobic incubation and a pre-diffusion step to avoid the diffusion inhibitory effect of the 0.07% agar in the thioglycolate broth. This method, referred to as the thioglycolate broth disk method, was compared with the agar dilution method. With clindamycin and chloramphenicol there was 100% correlation with the thioglycolate broth disk method and agar dilution method whereas penicillin showed a 95% agreement. The average overall agreement between the two methods was 94.5%.

The broth dilution method was developed by Stalons and Thornsberry (68) to simplify antibiotic susceptibility testing of anaerobic bacteria. Preliminary research indicated that neither the type of medium nor the type of anaerobic atmosphere had any significant effect on the action of the antibiotics. Different inoculum sizes, however, did produce variations in minimal inhibitory concentrations especially with the slow-growing anaerobes. Results indicated that chloramphenicol, clindamycin, tetracycline, erythromycin and lincomycin were the most effective against the majority of the 14 strains of anaerobic bacteria tested. Six strains of Bacteroides fragilis were the only anaerobic bacteria in the group to show resistance to penicillin-G and cephalothin. Vancomycin was ineffective against the B. fragilis strains and one strain of Fusobacterium nucleatum. B. thetaiotaomicron and Propionibacterium granulosum were the only anaerobes susceptible to gentamicin. This study and a study by Fass et al. (25) evaluated the three-tube categorization method. The method is an abbreviated version of the broth dilution method using only three antibiotic concentrations to determine the degree of susceptibility. In both studies the minimal inhibitory concentration values obtained by the broth dilution method correlated well with the values obtained with the three-tube categorization method.

By scaling down the broth dilution method, Rotilie et al. (62) devised a microdilution technique to determine the activity of eight antibiotics against 101 anaerobic isolates. The isolates

The broth dilution method was developed by Stalons and Thornsberry (68) to simplify antibiotic susceptibility testing of anaerobic bacteria. Preliminary research indicated that neither the type of medium nor the type of anaerobic atmosphere had any significant effect on the action of the antibiotics. Different inoculum sizes, however, did produce variations in minimal inhibitory concentrations especially with the slow-growing anaerobes. Results indicated that chloramphenicol, clindamycin, tetracycline, erythromycin and lincomycin were the most effective against the majority of the 14 strains of anaerobic bacteria tested. Six strains of Bacteroides fragilis were the only anaerobic bacteria in the group to show resistance to penicillin-G and cephalothin. Vancomycin was ineffective against the B. fragilis strains and one strain of Fusobacterium nucleatum. B. thetaiotaomicron and Propionibacterium granulosum were the only anaerobes susceptible to gentamicin. This study and a study by Fass et al. (25) evaluated the three-tube categorization method. The method is an abbreviated version of the broth dilution method using only three antibiotic concentrations to determine the degree of susceptibility. In both studies the minimal inhibitory concentration values obtained by the broth dilution method correlated well with the values obtained with the three-tube categorization method.

By scaling down the broth dilution method, Rotilie et al. (62) devised a microdilution technique to determine the activity of eight antibiotics against 101 anaerobic isolates. The isolates

included strains of Bacteroides, Clostridium, Fusobacterium, Peptococcus and Peptostreptococcus. Eighty-one percent and 88% of the strains were inhibited by tetracycline and minocycline at concentrations of 6.2 μg/ml and 1.6 μg/ml, respectively. Chloramphenicol inhibited 98% of all strains at concentrations of 12.5 μg/ml or less. Clindamycin at concentrations of 3.1 μg/ml or less inhibited 100% of the Bacteroides, Fusobacterium and anaerobic cocci, 95% of Clostridium perfringens and 77% of the other clostridia. Ampicillin at a concentration of 3.1 μg/ml inhibited only 32% of the Bacteroides fragilis strains whereas 100% of the other anaerobic bacteria were inhibited at this concentration. Carbenicillin at a concentration of 12.5 μg/ml and cephalothin at a concentration of 6.2 μg/ml inhibited all strains except certain strains of B. fragilis and clostridia. Gentamicin was generally inactive against the majority of strains tested. Steingrimsson et al. (69) further evaluated the microdilution method by comparing it to the agar dilution method. Six antibiotics were tested agsinst 80 strains of Bacteroides fragilis. All strains were sensitive to clindamycin and chloramphenicol, 15.9% were susceptible to ampicillin, 29.7% were susceptibile to tetracycline and only 4.1% were susceptible to penicillin. The minimal inhibitory concentrations determined by the microdilution and agar dilution methods were not significantly different.

The agar dilution method has been used by many investigators as a reference method in determining the antibiotic susceptibility

of anaerobic bacteria (1, 23, 44, 72, 74). Sutter and Finegold (73), using the agar dilution technique, determined the antibiotic sensitivity of 492 anaerobic bacteria. Penicillin-G at a concentration of 32 U/ml inhibited most of the anaerobic bacteria. However only 72% of the Bacteroides fragilis strains were susceptible to this concentration. At a concentration of 16 µg/ml, ampicillin and cephalothin inhibited 56% and zero percent of the B. fragilis strains, respectively. The majority of the other anaerobic bacteria were inhibited by this concentration. Chloramphenicol at a concentration achievable in the blood inhibited all anaerobic bacteria except for one strain of B. thetaiotaomicron and one strain of B. clostridiiformis subsp. clostridiiformis. All B. fragilis strains were susceptible to clindamycin at a concentration of 8 µg/ml. Resistant strains were three of the Bacteroides species, nine of the Peptococcus species and four of the Clostridium species. Tetracycline and doxycycline at concentrations greater than 64 µg/ml and 16 µg/ml, respectively, were required to inhibit most of the anaerobic bacteria. Doxycycline was more effective against the B. fragilis strains than tetracycline. Erythromycin showed poor activity against strains of B. fragilis; however all the Clostridium species and most of the Fusobacterium species were susceptible to a concentration of 8 µg/ml. Carbenicillin had excellent activity against all anaerobic bacteria tested with only five percent of the B. fragilis strains showing resistance.

Kimsey and Hirsh (40) reported the antibiotic susceptibility of various anaerobic bacteria isolated from animals. Results indicated that 90-95% of the isolates were susceptible to penicillin-G, tetracycline, clindamycin, chloramphenicol and ampicillin at concentrations achievable in the blood of the animals. The aminoglycosides, streptomycin, neomycin, kanamycin and gentamicin were totally ineffective against the isolates.

Beta-Lactamases in Anaerobic Bacteria

In recent years the relationship between beta-lactamase production by the Bacteroides fragilis group and their in vitro resistance to cephalosporins and penicillins has been studied (15, 46, 55, 63, 77). In regards to specific substrates hydrolyzed, beta-lactamases are classified as either penicillinases, cephalosporinases or both. In B. fragilis, cephalosporinases are not inducible by either penicillin or cephalothin but are inhibited by cloxacillin, clavulanic acid and p-chloromercuribenzoate. The exact location of the beta-lactamase enzyme within the bacterial cell has still not been resolved. It is believed however that the enzyme is either cell-bound or contained in the periplasmic space. Besides B. fragilis, distinct beta-lactamase enzymes are also produced by certain strains of B. ovatus, B. vulgatus, B. thetaiotaomicron, B. distasonis, B. melaninogenicus subsp. melaninogenicus, B. melaninogenicus subsp. intermedius, B. asaccharolyticus, B. oralis, Clostridium clostridiiformis (B. clostridiiformis subsp. clostridiiformis) and C. ramosum.

Several techniques have been developed recently to detect beta-lactamase activity in anaerobic bacteria. Weinrich and Del Bene (80), using an alkalimetric method, reported beta-lactamase activity in eight strains of Bacteroides fragilis. Rosenblatt and Neumann (61) tested penicillinase activity by an iodometric assay. This method used a penicillin-iodine mixture and detected penicillinase activity in 28 strains of Bacteroides melaninogenicus. Bourgault and Rosenblatt (13) identified beta-lactamase activity with a rapid, simple slide test referred to as the Nitrocefin test. This assay employs the chromogenic cephalosporin 87/112 as a substrate (54). Seventy-seven strains of Bacteroides fragilis and 25 strains of B. melaninogenicus which were resistant to penicillin also gave a positive Nitrocefin test. All Fusobacterium isolates were susceptible to penicillin and were negative for the Nitrocefin test.

Research Objectives

Anaerobic bacteria have long been known to cause a variety of human infections. Only recently anaerobic bacteria have been implicated as potential pathogens in specific animal diseases. Information concerning the role of anaerobic bacteria in animal infections is very limited, especially on the subject of anaerobic bacteria involved in bovine lung abscesses. To investigate the possibility of anaerobic bacteria being associated with bovine lung abscesses, research on this topic was divided into four parts.

In the first part, methods described by Holdeman and Moore (39) were used to isolate and identify anaerobic bacteria present in bovine lung abscesses. Additional information on incidence of facultatively anaerobic bacteria and location of abscesses was also recorded.

The second part of the research was devoted to determining the antibiotic susceptibility of the anaerobic bacteria isolated from bovine lung abscesses. In conjunction with antibiotic susceptibility testing, anaerobic bacteria were tested for beta-lactamase activity. This information might prove useful in treating cattle diagnosed as having a lung abscess.

In the third part, experiments were conducted to evaluate the effectiveness of phenethyl alcohol as a selective agent in PRAS roll tubes. This selective medium was developed in an effort to facilitate the recovery of anaerobic bacteria from polymicrobial infections.

In the past, most anaerobic bacteria were isolated and identified by methods described in the Virginia Polytechnic Institute (VPI) Anaerobe Laboratory Manual (39). Recently however, modifications of the VPI method have been developed to allow easier and faster identification of anaerobic bacteria. Bremmon (14) modified the VPI method and developed the South Dakota State University (SDSU) method for identification of anaerobic bacteria. The use of PRAS carbohydrate concentrates in the SDSU method of identifying anaerobic bacteria distinguishes it from the VPI method of identification.

In the fourth part of the research, experiments were conducted to evaluate the SDSU method by comparing the results of this method with results in the Anaerobe Laboratory Manual (39). These experiments had a three-fold purpose: 1) to determine the reliability of the SDSU PRAS carbohydrate concentrates for fermentation testing of anaerobic bacteria, 2) to test the quality of SDSU PRAS bacteriological media and 3) to evaluate the overall accuracy of the SDSU method for identifying anaerobic bacteria.

MATERIALS AND METHODS

Source of Specimens

All lung abscesses for cultural examination for anaerobic bacteria were obtained from approximately 2000 cattle processed on the "kill floor" of a local meat packing plant (Sioux Falls, SD).

Specimen Collection and Transport

Bovine lungs were inspected visually and by palpation. Any portions of a lung exhibiting firmness and a walled-off area were considered abscessed and collected. Lungs with pneumonia were not collected. Lung abscesses were collected 15 min after the death of the animal and within 2 min after evisceration. Healthy lung tissue was also collected as a control.

Abscesses were carefully trimmed away from normal lung tissue in order to avoid cutting through the abscess wall. A data report form was filled out for each abscess collected; it included area of lung infected and the specimen size. Each abscess was placed in a Whirl-Pak bag (Scientific Products, McGraw Park, Ill.), labelled to correspond with the data report form, and transported to the laboratory. During collection and transportation, all specimens were maintained at room temperature (21 to 26°C) until processed at the laboratory.

Processing Specimens

At the laboratory, lung abscesses were removed one at a time from the Whirl-Paks and pinned onto a dissecting board. The exterior of each abscess was seared several times with a hot spatula to sterilize the outer tissue. The abscess was lanced with an alcohol-sterilized scalpel (Scientific Products). A small portion of the abscess wall and exudate, approximately 1 to 2 g, was aseptically removed and anaerobically transferred to a 10-ml PRAS dilution blank (39) containing eight glass beads (#G600, 6-mm solid glass beads, Scientific Products). Each dilution blank was mixed at high speed for 5 min with a Vortex mixer (Scientific Products).

Media

All pre-reduced, anaerobically sterilized (PRAS) media were prepared as described in the Anaerobe Laboratory Manual (39, 41). PRAS media used in this study included: chopped meat, chopped meat-carbohydrate, chopped meat agar slants, brain heart infusion agar supplemented with 0.5% yeast extract (BHIA-S) roll tubes, dilution blanks, gelatin, peptone yeast extract (PY), peptone yeast extract glucose (PYG), PYG without resazurin, PYG agar deeps, milk, esculin, tryptone yeast extract and brain heart infusion broth supplemented with 0.5% yeast extract (BHI-S). All PRAS media except dilution blanks were supplemented with vitamin K_1 (1 μg/ml) and hemin (5 μg/ml).

PRAS carbohydrate concentrates were prepared as described by Bremmon (14). Twenty percent carbohydrate concentrates included

cellobiose, fructose, galactose, gluconate, glucose, inositol, lactose, maltose, mannitol, mannose, raffinose, rhamnose, salicin, sorbitol, starch, sucrose and xylose. Ten percent carbohydrate concentrates included adonitol, amygdalin, arabinose, erythritol, glycogen, melezitose, melibiose, ribose and trehalose. Bile and polyoxyethylene sorbitan mono-oleate (Tween-80) were also prepared in PRAS concentrated form. All solutions were sterilized in 20 x 150 mm screw-capped test tubes by autoclaving for 15 min at 121°C (15 psi).

PRAS media were stored in the dark at room temperature to prevent resazurin breakdown and oxygen infusion.

Indol-Nitrite Medium was aerobically prepared and dispensed into 13 x 100 mm screw-capped test tubes. Tubes were sterilized by autoclaving for 15 min at 121° (15 psi).

Sources of Carbohydrates and Bacteriological Media Used

Galactose, glucose, inositol, mannitol, raffinose, sorbitol, sucrose, trehalose and xylose were obtained from Difco Laboratories (Difco), Detroit, Mich. Adonitol, amygdalin, arabinose, erythritol, esculin, fructose, gluconate, glycogen, maltose, mannose, melezitose, melibiose, rhamnose and salicin were obtained from the Sigma Chemical Co. (Sigma), St. Louis, Mo. Starch was obtained from Matheson, Coleman and Bell (MC/B), Norwood, Ohio. Cellobiose, lactose and ribose were supplied by Grand Island Biological Co. (GIBCO), Madison, Wisc.; Pfanstiehl Chemical Co., Waukegan, Ill.; and Eastman Kodak Co., Rochester, N.Y., respectively.

Bacteriological media supplied by Difco included: peptone, gelatin, indol-nitrite, oxagall (bile) and tryptone. Media obtained from GIBCO included: yeast extract, agar, brain heart infusion broth and brain heart infusion agar. Trypticase was supplied by Becton, Dickinson and Co., Cockeysville, Md. Whole milk was obtained from the SDSU Dairy Science Department. Ground beef was supplied by the SDSU Meat Laboratory.

Media components and additives supplied by MC/B included resazurin and Tween-80. Vitamin K_1, hemin and cysteine hydrochloride were obtained from Sigma.

Culture Techniques

An anaerobic culture system was constructed according to the Anaerobe Laboratory Manual (39). This system was used for all anaerobic manipulations including: transferring anaerobic cultures, rapid inoculation of differential media, streaking roll tubes and antibiotic susceptibility testing.

Platinum inoculating loops and platinum picking needles were used for all culture work to avoid oxidizing the reduced media. Platinum wire was supplied by the Carolina Biological Co., Burlington, N.C.

Kopeloff Gram Staining Method

The Kopeloff-Beerman modification of the Gram Stain was used for all Gram staining. This procedure can be found in the

Manual of Microbiological Methods (66) and the Anaerobe Laboratory Manual (39).

Isolation of Anaerobic Bacteria From Bovine Lung Abscesses

Streaking for Isolation. Using an inoculating loop, the material in each dilution blank was Gram stained and anaerobically streaked on a BHIA-S roll tube. Roll tubes were incubated at 37°C for 24 to 72 h.

Colony Picking. BHIA-S roll tubes containing isolated colonies were placed on a plastic prop and viewed under a stereoscopic dissecting microscope, 30x, (American Optical Co., Buffalo, N.Y.). Colonies displaying distinct morphological differences were described and marked. Marked colonies were anaerobically picked from each roll tube with a picking needle and transferred to chopped meat medium. Chopped meat cultures were incubated at 37°C for 24 to 48 h then Gram stained. Any area of confluent growth on each roll tube was Gram stained to detect any bacteria not present as isolated colonies. If all bacteria were not isolated, the area of confluent growth was restreaked.

Aerotolerance Testing. An aerotolerance test was performed on all bovine lung abscess isolates. One loopful of each 24 to 48 h chopped meat culture was aerobically streaked on 15 x 100 mm petri plates (Scientific Products) containing brain heart infusion agar with 5% defibrinated sheep blood and 0.5% yeast extract. Plates

were inverted, placed in a candle jar and incubated at 37°C for 72 h. Isolates showing growth after 72 h were considered facultatively anaerobic bacteria and not identified. Isolates classified as obligately anaerobic bacteria (anaerobic bacterial isolates) were subcultured to chopped meat medium for further identification.

Maintenance of Anaerobic Bacterial Isolates

During the process of identification, isolates were maintained in chopped meat medium at room temperature. Cultures were anaerobically transferred every three weeks into chopped meat medium and incubated at 37°C for 48 h. Cultures were Gram stained to monitor purity and growth.

Identification of Anaerobic Bacterial Isolates to the Genus Level

Isolates were identified to the genus level using standard anaerobic identification procedures (39). This involved observation of pigment formation, colonial morphology, Gram Stain, cellular morphology, heat test for spore formation, production of catalase and gas-liquid chromatographic analysis.

Heat Test for Spore Formation. Using a 9-inch Pasteur pipet (Scientific Products), ten drops (0.25 ml) of the starch concentrate was anaerobically transferred to a 5-ml PY broth tube. The starch broth was anaerobically inoculated with a chopped meat culture suspected of having spores. The starch broth tube with a clamped stopper was heated at 80°C for 10 min in a water bath. A control

tube, containing a volume of water equal to the volume of the starch broth, was used to monitor the water bath temperature. After heating, tubes were incubated at 37°C for 24 h. Any tubes showing turbidity after incubation were recorded as positive (spores present).

Catalase Test. Isolates were tested for catalase activity using the method described by Hansen and Stewart (34). A stock solution of Tween-80 was prepared by mixing 10 ml of Tween-80 and 90 ml of distilled water in a 125-ml Erlenmeyer flask and heating in a 60°C water bath until dissolved. A 15% hydrogen peroxide solution was prepared from 30% hydrogen peroxide and the Tween-80 stock solution (15% H_2O_2Tw80), and stored at 4°C.

Ten drops of 15% H_2O_2Tw80 and 10 drops of a 24 to 48 h chopped meat culture were added to a 12 x 75 mm test tube. Tubes were observed over a 30-min period for continuous bubbles. Control cultures with known positive catalase activity which were tested were Bacteroides fragilis SLCH and Propionibacterium acnes SLCH. Control cultures with known negative catalase activity which were tested were Fusobacterium nucleatum VPI-8025C and Bifidobacterium eriksonii VPI-1934.

Gas-Liquid Chromatographic Analysis. Twenty-four to 48 h chopped meat cultures were anaerobically inoculated into PYG medium. PYG cultures were incubated at 37°C for 36 h or until good growth appeared. Two ml of each PYG culture were withdrawn and 1 ml pipetted into each of two 12 x 75 mm test tubes. Tube #1 was

labelled for volatile fatty acid analysis. Tube #2 was labelled for non-volatile fatty acid analysis.

A standard procedure was used for analysis of volatile fatty acids and alcohols (39). Each 1-ml culture was acidified with 0.2 ml of 50% sulfuric acid (Mallinckrodt, St. Louis, Mo.). One ml of ethyl ether (MC/B) and 0.4 grams of sodium chloride (Mallinckrodt) were added to each acidified culture. Tubes were stoppered with rubber stoppers, inverted 20 times and centrifuged at high speed for 5 min. The ether layer was carefully pipetted off the aqueous layer and transferred to a second 12 x 75 mm test tube. Anhydrous magnesium sulfate (Mallinckrodt) was added to the ether. Tubes were stoppered and allowed to stand at room temperature for 5 to 10 min. A teflon-tipped, plunger type, 50-µl glass syringe (Unimetrics Corporation, Anaheim, Calif.) was used to remove a 14-µl quantity of the ether extract. The 14-µl sample was injected into the column of a gas-liquid chromatograph.

Analysis of methyl derivatives of pyruvic, lactic, fumaric and succinic acids was accomplished by standard procedures (39). Two ml of methanol (Fisher Scientific Co., Pittsburgh, Pa.) and 0.4 ml of 50% sulfuric acid were added to each 1-ml culture in tube #2. Tubes were stoppered with rubber stoppers, mixed by inverting and heated at 60°C in an aluminum temperature block for 30 min. After heating, 1 ml of water and 0.5 ml of chloroform (Fisher Scientific Co.) were added to each tube and the solutions were mixed by inverting 20 times. Tubes were centrifuged at high speed for

2 to 3 min. A 50-µl glass syringe was used to remove a 14-µl quantity of the chloroform extract which was layered directly under the aqueous phase. After wiping off the outside of the injector needle, the 14-µl sample was injected into the column of a gas-liquid chromatograph.

The gas-liquid chromatograph used for all analyses was the Dohrmann Anaerobic Bacteriology System (Model 15C-3) distributed by Clinical Analysis Products Co. (CAPCO), Sunnyvale, Calif. This chromatograph was used in conjunction with a Dohrmann potentiometric strip recorder (Model R-300, CAPCO) and a matched pair of detector elements (Model 1011, CAPCO). The column used in this system was 0.25 in x 6 ft stainless steel and packed with 15% Sp-1220/1% H_3PO_4 on 100/120 Chromosorb W-AW (Supelco, Bellefonte, Pa.).

Chromatographic conditions were as follows: detector temperature (135°C), column temperature (125°C), injection temperature (150°C), attenuation (1-4), recorder speed (low), detector current (120 ma) and a helium flow rate of 120 cc/min at a gauge pressure of 30 psi.

Volatile fatty acid, non-volatile fatty acid and alcohol standard were provided by Supelco. Each standard was run once a week to determine the various peak positions and the amounts of each component. Uninoculated PYG medium was chromatographed to ascertain the amount of volatile and non-volatile fatty acids present in the medium.

Identification of Anaerobic Bacterial Isolates to the Species Level

Isolates identified to the genus level were further characterized by performing biochemical and physiological tests described in the Anaerobe Laboratory Manual (39).

Preparation, Inoculation, Incubation and pH Determination of Carbohydrate Fermentation Tests. The average volume of 10 drops dispensed vertically from a 9-inch Pasteur pipet was determined to be approximately 0.25 ml by weighing a known quantity of drops.

Ten drops (0.25 ml) of each 10% PRAS carbohydrate concentrate were anaerobically transferred to 5-ml PY broth (basal medium) tubes. This gave a final concentration of 0.5% in the carbohydrate fermentation broths. One percent carbohydrate fermentation broths were prepared by anaerobically dispensing 10 drops (0.25 ml) of each 20% PRAS carbohydrate concentrate into 5-ml basal medium tubes.

Carbohydrate fermentation broths were anaerobically inoculated with 5 drops of a 24 to 48 h chopped meat culture. Control tubes containing 5 ml of basal medium without carbohydrates were also inoculated at this time. Inoculum was dispensed by a rapid multiple inoculating device. This apparatus was part of the anaerobic culture system.

Inoculated tubes were incubated at 37°C for 24 h. Incubation time was extended to 48 h for any isolate showing poor growth.

The pH of each fermentation broth culture was determined by using a Corning Model 130 pH meter and a combination-electrode

(Cat. 476051, Corning Scientific Instruments, Medfield, Mass.). Before interpreting pH readings, the pH of each fermentation broth culture was compared with the pH of the control culture. The pH of control cultures ranged from 6.1 to 6.5. Fermentation was determined as positive or negative for each tube by the pH standards used by Holdeman and Moore (39). A pH of 6.1 and above was recorded as negative (-). A pH of 5.5 to 6.0 was recorded as weak acid (w). A pH below 5.5 was recorded as strong acid (a).

Additional Biochemical and Physiological Tests. Unless otherwise mentioned, biochemical and physiological tests were inoculated and incubated in the same manner as the carbohydrate fermentation broths.

Esculin hydrolysis was determined by adding 2 to 3 drops of ferric ammonium citrate solution to a culture in esculin medium. Development of black color within 5 min was recorded as a positive test.

PYG cultures were used to test for ammonia production. Two drops of culture and 4 drops of Nessler's solution were added to a white spot plate (Scientific Products). Appearance of an orange color within 30 sec was recorded as a positive test. Uninoculated PYG medium was also tested and served as a control.

Starch hydrolysis was detected in starch broth cultures by adding 3 drops of Gram's iodine. Development of a blue-black color within 5 sec was recorded as negative hydrolysis while absence of color was recorded as positive hydrolysis.

Cultures grown in PYG (without resazurin) medium were tested for acetylmethylcarbinol (AMC) production. One ml of culture was mixed with 10 drops AMC solution A and 5 drops AMC solution B (39). Tubes were unstoppered and observed over a 15-min period. Development of red color was recorded as a positive test. Uninoculated medium was used as a control.

Bile stimulation tests were performed by comparing growth in PYG medium with growth in bile medium (PYG medium with 2% bile). Growth in bile medium was recorded as either negative (inhibited) or positive (1+ to 4+). Cultures growing better in bile medium than in PYG medium were recorded as stimulated (S).

Gelatin cultures (incubated at 37°C for 72 h) and an uninoculated gelatin tube (control) were refrigerated until the control tube solidified. Tubes were removed to room temperature and gelatin cultures which had failed to solidify were recorded as positive for gelatinase. Gelatin cultures liquefying in less than $\frac{1}{2}$ the time required for the control tube were recorded as weak positive.

Motility was checked by placing one drop of a 12 to 24 h chopped meat culture on a slide, adding a coverslip and viewing under an oil-immersion lens.

Tryptone yeast extract cultures were tested for indole production. Fifteen drops of xylene were added to 2 ml of culture and mixed well. Ten drops of Ehrlich's reagent were slowly added to each tube and the tubes observed over a 30-min period. Appearance of a red color was recorded as a positive test.

Chopped meat cultures were incubated at 37°C for 2 weeks and observed for meat digestion. Presence of a fluffy powder was recorded as positive digestion (+).

Milk cultures were incubated at 37°C for 14 days and checked for curd formation (c), digestion (d) and acid production (a).

PYG agar deeps were used to demonstrate gas production. Molten PYG agar was cooled to 47°C, inoculated and covered with flamed aluminum foil. Gas production was recorded as follows: bubbles in area of growth (+), small splits in the agar (++), agar raised halfway up the tube (+++) and agar raised to the top of the tube (++++).

Twenty drops of Nitrite Reagent A and 10 drops of Nitrite Reagent B were added to cultures in Indol-Nitrite Medium (39). Development of a red color indicated positive nitrate reduction. If no color developed within 10 min, a pinch of zinc dust was added. After adding zinc dust, appearance or absence of a red color was recorded as negative and positive reactions, respectively.

Antibiotic Susceptibility Testing of Anaerobic Bacterial Isolates

The modified broth-disk method was used for testing the antibiotic susceptibility of anaerobic bacterial isolates (39, 82). Isolates were tested against 10 different antibiotics.

The test concentrations of antibiotics used are as follows:

Antibiotics	No. disks per tube (5 ml)	Test concentration
Penicillin-G (Difco)	1	2 units/ml
Ampicillin (Difco)	2	4 µg/ml
Carbenicillin (Difco)	5	100 µg/ml
Cephalothin (Difco)	1	6 µg/ml
Doxycycline (Difco)	1	6 µg/ml
Tetracycline (Difco)	1	6 µg/ml
Clindamycin (Difco)	4	1.6 µg/ml
Chloramphenicol (Difco)	2	12 µg/ml
Erythromycin (Difco)	1	3 µg/ml
Vancomycin (Difco)	1	6 µg/ml

For each antibiotic the specified number of disks were anaerobically added to 5-ml BHI-S broth tubes. This simulates the blood level concentration achievable by a specific antibiotic. Using a Pasteur pipet, one drop of a 24 to 36 h chopped meat-carbohydrate culture was anaerobically transferred to each BHI-S broth tube containing antibiotic disks. A control tube, 5-ml BHI-S broth tube without antibiotics, was also inoculated at this time. Tubes were incubated at 37°C for 18 to 24 h. After incubation, the turbidity of the antibiotic tubes was visibly compared to that of the inoculated control tube not containing antibiotics. An anaerobic bacterial isolate was recorded as resistant (R) to an antibiotic if the antibiotic tube had a turbidity 50% or greater than the turbidity of the control tube. An anaerobic bacterial isolate was recorded as susceptible (S) to an antibiotic if the antiobiotic tube had no visible turbidity. An anaerobic bacterial isolate was recorded as indeterminate (I) if the antibiotic tube had turbidity equal to 50% of the turbidity in the control tube.

Source and Maintenance of Stock Cultures

Obligately anaerobic bacteria in the stock culture collection of the SDSU Microbiology Department were originally acquired from two sources. Actinomyces viscosus VPI-7596, Bacteroides vulgatus VPI-8811, Bifidobacterium adolescentis VPI-C489, B. eriksonii VPI-1934, Clostridium chauvoei VPI-1527, C. sordelli VPI-5917, Eubacterium lentum VPI-1947B, Fusobacterium nucleatum VPI-8025C, Peptostreptococcus anaerobius VPI-5750, Propionibacterium freudenreichii subsp. shermanii VPI-0405, Streptococcus constellatus VPI-3810, S. morbillorum VPI-5424 and Veillonella parvula VPI-8588 were obtained from the Anaerobe Laboratory at Virginia Polytechnic Institute and State University (VPI), Blacksburg, Va. Bacteroides asaccharolyticus SLCH, B. fragilis SLCH, Clostridium perfringens SLCH, C. sporogenes SLCH, C. tertium SLCH, Peptococcus magnus SLCH, Propionibacterium acnes SLCH were supplied by T. Brotherton, Microbiology Laboratory, St. Louis Children's Hospital (SLCH), St. Louis, Mo.

Cultures were maintained in chopped meat medium at room temperature. Cultures were anaerobically transferred every month to chopped meat medium and incubated at 37°C for 48 h. Cultures were Gram stained to check growth and purity.

Facultatively anaerobic bacteria used in this study were acquired from the stock culture collection of the SDSU Microbiology Department. The cultures included Edwardsiella tarda, Citrobacter sp., Escherichia coli, Enterobacter aerogenes, Klebsiella pneumonia,

Proteus mirabilis, P. morganii, P. vulgarius, Salmonella arizonae, Serratia marcescens, Shigella dysenteriae, Bacillus cereus, Corynebacterium pyogenes, C. diphtheriae, Listeria monocytogenes, Staphylococcus epidermidis, S. aureus, Streptococcus agalactiae, S. faecalis, S. fecium, S. pyogenes, S. uberis and S. bovis.

Cultures were maintained on aerobic BHIA-S slants at 4°C. Cultures were transferred every month to BHIA-S slants and incubated at 37°C for 48 h or until good growth appeared. Cultures were Gram stained to check purity.

Determination of Beta-Lactamase Activity in Anaerobic Bacteria

A rapid, simple spot plate test, using the chromogenic cephalosporin 87/112 (Nitrocefin, Glaxo) as a substrate, was developed to detect beta-lactamase activity. This test is similar to the slide test developed by Bourgault and Rosenblatt (13). Nitrocefin was supplied by C. H. O'Callaghan, Microbiology Division, Glaxo Research Ltd., Greenford, England.

A working solution of Nitrocefin was prepared by adding 0.5 ml of dimethyl sulfoxide (Mallinckrodt) to 5 mg of Nitrocefin powder in a 20 x 150 mm test tube. Immediately after the powder had dissolved, 9.5 ml of 0.1 M phosphate buffer pH 7.0 was added and the solution mixed. The final concentration of Nitrocefin was 500 µg/ml. The solution was dispensed in 0.5-ml portions into 10 x 75 mm test tubes, stoppered and refrigerated (4°C) for up to 14 days.

Anaerobic bacterial isolates and Bacteroides spp. from the stock culture collection were tested for beta-lactamase activity. Four drops of a 48 h chopped meat carbohydrate culture were mixed with 3 drops of the Nitrocefin solution on a white spot plate. A change in color from yellow to red within 30 min was recorded as a positive reaction. Uninoculated chopped meat carbohydrate medium was also tested and served as a control.

Results of the beta-lactamase test were correlated with beta-lactam antibiotic susceptibility. Beta-lactam antibiotics used in this study were penicillin-G, ampicillin, carbenicillin and cephalothin. Antibiotics were tested by the modified broth-disk method (82).

Use of Phenethyl Alcohol in PRAS Roll Tubes for Isolation of Obligately Anaerobic Bacteria

BHIA-S roll tubes supplemented with six different concentrations of phenethyl alcohol (MC/B) were used to evaluate the effectiveness of phenethyl alcohol (PEA) as a selective agent. The medium was referred to as PRAS PEA roll tubes and prepared according to the Anaerobe Laboratory Manual (39). The composition of the medium is as follows:

PRAS PEA roll tubes

brain heart infusion broth	3.7 g
yeast extract	0.5 g
resazurin solution	0.4 ml
cysteine hydrochloride	0.05 g
hemin solution	1.0 ml
vitamin K_1	0.02 ml
distilled water	100 ml

Anaerobic bacterial isolates and Bacteroides spp. from the stock culture collection were tested for beta-lactamase activity. Four drops of a 48 h chopped meat carbohydrate culture were mixed with 3 drops of the Nitrocefin solution on a white spot plate. A change in color from yellow to red within 30 min was recorded as a positive reaction. Uninoculated chopped meat carbohydrate medium was also tested and served as a control.

Results of the beta-lactamase test were correlated with beta-lactam antibiotic susceptibility. Beta-lactam antibiotics used in this study were penicillin-G, ampicillin, carbenicillin and cephalothin. Antibiotics were tested by the modified broth-disk method (82).

Use of Phenethyl Alcohol in PRAS Roll Tubes for Isolation of Obligately Anaerobic Bacteria

BHIA-S roll tubes supplemented with six different concentrations of phenethyl alcohol (MC/B) were used to evaluate the effectiveness of phenethyl alcohol (PEA) as a selective agent. The medium was referred to as PRAS PEA roll tubes and prepared according to the Anaerobe Laboratory Manual (39). The composition of the medium is as follows:

PRAS PEA roll tubes

brain heart infusion broth	3.7 g
yeast extract	0.5 g
resazurin solution	0.4 ml
cysteine hydrochloride	0.05 g
hemin solution	1.0 ml
vitamin K_1	0.02 ml
distilled water	100 ml

Phenethyl alcohol was added at concentrations of 0.05%, 0.10%, 0.15%, 0.20%, 0.25% and 0.30% (vol/vol). The pH of each medium was adusted with 8 N sodium hydroxide (Mallinckrodt) to a pre-autoclave value of 6.8 and 10 ml dispensed into 25 x 142 mm anaerobe tubes (Bellco Glass Inc., Vineland, N.J.) containing 0.25 g of agar. Media were autoclaved for 15 min at 121°C (15 psi). The pH of the media after autoclaving ranged from 6.8 to 7.2.

Facultatively anaerobic bacteria and obligately anaerobic bacteria from the stock culture collections and anaerobic bacterial isolates were anaerobically transferred to chopped meat-carbohydrate medium and incubated at 37°C for 24 to 48 h. One loopful of a chopped meat-carbohydrate culture was anaerobically streaked on 0.05%, 0.10%, 0.15%, 0.20%, 0.25%, 0.30% PEA roll tubes and a BHIA-S roll tube without PEA (control tube). All tubes were incubated at 37°C. Growth in PEA roll tubes was checked at 24, 48, 72 and 96 h of incubation and compared with the growth in the control tube. The effects of PEA on culture growth were recorded as follows: no inhibition (++++), slight inhibition (+++), moderate inhibition (++), extreme inhibition (+) and complete inhibition (-).

Comparison of the Results of Two Methods for the Identification of Obligately Anaerobic Bacteria

Twenty obligately anaerobic bacteria from the stock culture collection were used to evaluate the SDSU method of identification. Genera represented by the stock culture collection include: Bacteroides, Fusobacterium, Clostridium, Bifidobacterium,

Propionibacterium, Peptostreptococcus, Actinomyces, Peptococcus, Streptococcus, Eubacterium and Veillonella. Each genus was further differentiated by gas-liquid chromatographic analysis and anaerobic inoculation of the appropriate PRAS biochemical media designated in the Anaerobe Laboratory Manual (39). All biochemical tests, physiological tests, PRAS carbohydrate concentrate tests, gas-liquid chromatographic analyses and Gram Stains were performed by the SDSU method previously described for the identification of anaerobic bacterial isolates.

Results of the SDSU method were compared with the results of the VPI method for each test performed and were used to determine the overall accuracy of the SDSU method of identification. Results for the VPI method were obtained from the Anaerobe Laboratory Manual (39).

RESULTS AND DISCUSSION

The principal objective of this study was to determine the relative incidence, species and antibiotic susceptibility of obligately anaerobic bacteria found in bovine lung abscesses.

Description of Specimens Collected for Anaerobic Culture Examination

From the 2000 cattle examined, a total of 15 lung abscesses were collected for anaerobic culture examination. Each abscess was collected from a different animal. The approximate size and location (lobe infected) of each abscess are shown in Table 1. The abscesses ranged in size from 1 to 9 cm with an average diameter of approximately 4 cm. Finegold (27), in order to distinguish lung abscess from necrotizing pneumonia, has defined a lung abscess as a cavity at least 2 cm in diameter. Only 2 of the 15 abscesses collected in this study were not in compliance with this definition. In regard to location, 12 (80%) of the 15 abscesses occurred in the right and left diaphragmatic lobes (Table 2). Two (13%) of the abscesses occurred in the right upper apical lobe. One abscess was collected from the left cardiac lobe.

Lung abscesses, classified as pleuropulmonary infections, are usually caused by the aspiration of oropharyngeal secretions (30). In cattle, Bacteroides melaninogenicus, Fusobacterium necrophorum, Eubacterium spp., Actinomyces bovis, Peptococcus indolicus, Corynebacterium pyogenes and Pasteurella multocida are just a few of

Table 1. Bovine lung abscesses collected for anaerobic culture examination[a]

Specimen no.	Location of abscess	Approx diam of abscess (cm)
11	Right diaphragmatic lobe	4
15	Left diaphragmatic lobe	2
19	Left diaphragmatic lobe	4
20	Left diaphragmatic lobe	3
21	Right diaphragmatic lobe	2
22	Right upper apical lobe	5
23	Right diaphragmatic lobe	1
26	Left diaphragmatic lobe	5
27	Right diaphragmatic lobe	5
39	Right diaphragmatic lobe	9
40	Right upper apical lobe	4
41	Left cardiac lobe	3
42	Right diaphragmatic lobe	2
43	Left diaphragmatic lobe	1
44	Left diaphragmatic lobe	8

[a]Each abscess was collected from a different animal.

the bacteria composing the normal flora of the oral cavities (9, 45, 65, 67, 86). Thus in cattle, aspiration of oropharyngeal secretions produces massive contamination of the lower respiratory tract by a complex flora of organisms. Besides aspiration, bacteria can also enter the bovine lung in a variety of other ways: 1) by extension from a nearby infection, 2) via the blood stream from a distant infection, e.g., liver abscess and 3) by inhalation of infective droplets.

The aspiration of oropharyngeal secretions and the in situ position of bovine lungs may help to explain the high rate of abscess formation in the diaphragmatic lobes (Table 2). In the standing position of cattle, the in situ position of the lung is such that lower areas of the diaphragmatic lobes, most of the apical lobes and all of the cardiac lobes are below the thoracic inlet and are considered ventral parts of the lung. By aspiration and gravitational pull, infective organisms from oropharyngeal secretions can drain into ventral bronchioles, alveolar ducts and alveoli. Obligately anaerobic bacteria will proliferate in these areas if the oxidation-reduction potential is lowered by 1) impairment of local blood supply by trauma to blood vessels, shock and edema, 2) tissue devitalization from infection and malignancy and 3) growth of aerobic and facultatively anaerobic bacteria (27). The fulfillment of any one of these predisposing factors could eventually result in abscess formation by anaerobic bacteria.

Table 2. Distribution of abscesses collected from 15 bovine lungs[a]

Location of abscess	No. of specimens (%)
Right diaphragmatic lobe	6 (40)
Left diaphragmatic lobe	6 (40)
Right upper apical lobe	2 (13)
Left cardiac lobe	1 (7)

[a]Determined from Table 1.

Table 3. Results obtained from anaerobic culture examination of 15 bovine lung abscesses

Type of bacteria	No. of specimens (%)
No significant growth	3 (20)
Obligately anaerobic bacteria only	3 (20)
Facultatively anaerobic bacteria only	3 (20)
Facultatively and obligately anaerobic bacteria	6 (40)

Incidence of Anaerobic Bacteria in Bovine Lung Abscesses

Facultatively and obligately anaerobic bacteria were present together in 6 (40%) of the 15 abscesses culturally examined (Table 3). Only 3 (20%) of the abscesses contained obligately anaerobic bacteria in pure culture. Since obligately anaerobic bacteria were found more times in mixed culture than in pure culture, the question arises as to whether these organisms are primary or secondary invaders. An organism is considered a secondary invader if it requires a previous infection, wound, or other predisposing factor to gain entry into the host. Three abscesses did not yield any facultatively or obligately anaerobic bacteria; however, these abscesses were not classified as sterile since an aerobic plate was not included in the initial isolation procedure.

For a control, healthy lung tissue was collected from right diaphragmatic and right cardiac lobes of two different animals. Regardless of the location, healthy lung tissue failed to produce any growth after anaerobic culture examination.

Identification of Anaerobic Bacterial Isolates

The biochemical and physiological reactions and gas-liquid chromatographic analysis for each anaerobic bacterial isolate are shown in Table 4. A total of 19 isolates were obtained from the 9 abscesses containing obligately anaerobic bacteria. Each abscess yielded an average of 2 anaerobic species (range 1-5).

Table 4. Identification of anaerobic bacterial isolates: Physiological and biochemical characteristics[a]

Isolate[b]	Biochemical and physiological tests[c]																														
	Adonitol	Amygdalin	Arabinose	Cellobiose	Erythritol	Esculin pH	Esculin hyd.[e]	Fructose	Gelatinase	Glucose	Glycogen	Inositol	Lactose	Maltose	Mannitol	Mannose	Melibiose	Raffinose	Rhamnose	Ribose	Salicin	Sorbitol	Starch pH	Starch hyd.[f]	Sucrose	Trehalose	Xylose	AMC[g]	Ammonia		
(11)-2	*	*	-	-	*	-	-	-	-	-	*	-	-	-	-	*	*	-	*	*	-	*	-	-	-	-	-	-	-		
(11)-5	*	-	*	*	-	-	-	a	-	a	-	-	a	w	-	a	w	a	-	-	*	-	-	-	a	*	-	-	+		
(15)-1	*	-	*	*	-	-	-	-	w	-	-	-	-	-	-	-	-	-	-	-	*	*	-	-	-	*	-	-	+		
(19)-1	*	-	*	*	-	-	-	-	w	-	-	-	-	-	-	-	-	-	-	-	*	*	-	-	-	*	-	-	+		
(22)-2	*	-	*	*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	*	-	-	-	*	-	-	+		
(22)-4	*	*	*	-	*	-	-	*	*	w	*	*	-	-	*	-	*	*	*	*	*	-	-	-	w	*	-	-	+		
(22)-6	*	-	*	*	-	-	-	-	-	-	-	-	-	-	-	-	-	w	w	-	-	*	*	-	-	-	*	-	-	-	
(26)-1	w	*	*	*	*	*	-	-	a	*	a	*	*	w	w	a	*	*	*	*	*	*	a	*	*	a	a	*	-	+	
(26)-3	*	-	*	*	-	-	-	-	-	-	-	-	-	w	w	-	-	-	-	-	w	*	*	-	-	-	*	-	-	+	
(27)-1	*	-	*	*	-	-	-	w	w	-	-	-	-	-	-	-	-	-	-	-	*	*	-	-	w	*	-	-	+		
(39)-3	*	-	*	*	-	-	-	a	-	a	-	-	w	w	-	a	-	a	-	-	*	-	-	-	a	*	-	-	-		
(39)-4	*	*	*	-	-	-	*	*	*	-	*	*	-	-	*	-	*	*	*	*	*	-	-	-	-	*	-	-	+		
(42)-1	*	-	*	*	-	-	-	-	w	-	-	-	-	-	-	-	-	-	-	-	*	*	-	-	w	*	-	-	+		
(42)-2[k]	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		

Table 4. (Continued)

Bile growth / Catalase[h] / Gas prod. / Indole / Meat dig.[i] / Milk / Motility / Nitrate red.[j]	GLC analysis[d]	Identity
4 - 1 - - * - -	Safpibiv	Bacteroides sp.
* - - - - a - -	AFLs	Eubacterium aerofaciens
* - 1 + + d - -	Bafps	Fusobacterium necrophorum
* - 1 + + d - -	Bafps	F. necrophorum
* - 4 + - - - -	Bafpls	F. gonidiaformans
* - 3 + - * - +	ABfps	Peptococcus indolicus
* - 3 + - d - -	ABfpls	Fusobacterium necrophorum
* + 1 + - * - +	APLs	Propionibacterium acnes
* - 2 + + d - -	ABfps	Fusobacterium necrophorum
* - 1 + - d - -	ABfpls	F. necrophorum
* - 2 - - a - -	AFLs	Eubacterium aerofaciens
* - 3 + - * - +	ABfps	Peptococcus indolicus
* - 4 + + d - -	Bapls	Fusobacterium necrophorum
* * * * * * * *	Sapivl	Bacteroides sp.

Table 4. (Continued)

Isolate	Biochemical and physiological tests																														
	Adonitol	Amygdalin	Arabinose	Cellobiose	Erythritol	Esculin pH	Esculin hyd.	Fructose	Gelatinase	Glucose	Glycogen	Inositol	Lactose	Maltose	Mannitol	Mannose	Melibiose	Raffinose	Rhamnose	Ribose	Salicin	Sorbitol	Starch pH	Starch hyd.	Sucrose	Trehalose	Xylose	AMC	Ammonia		
(44)-1	*	-	*	*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	*	-	-	-	*	-	-	+		
(44)-2	*	*	-	w	*	-	-	a	-	a	*	-	a	w	w	*	*	a	*	*	w	*	w	-	w	w	w	-	-		
(44)-3	*	*	*	-	*	-	-	*	*	-	*	*	-	-	*	-	*	*	*	*	*	-	-	-	-	*	-	-	+		
(44)-5	*	-	*	*	-	-	-	-	w	-	-	-	-	-	-	-	-	-	-	-	*	*	-	-	-	*	-	-	+		
(44)-6	*	-	*	*	-	-	-	a	w	a	-	-	a	w	-	a	-	a	-	w	*	-	-	-	a	*	-	-	+		

[a] Identification determined by the method of Holdeman and Moore (39).

[b] Numbers represent the specimen ( ), Table 1, and the isolate.

[c] Abbreviations and symbols for reactions: a, acid (pH below 5.5); w, weak reaction or pH between 5.5 and 6.0; -, negative reaction; +, positive reaction; d, digestion; *, not tested; and numbers (bile growth and gas production), amount estimated on a "- to 4+" scale. A superscript indicates some strains react differently.

[d] Gas-liquid chromatographic (GLC) analysis was used to detect acids and alcohols produced in PYG broth cultures. Capital letters indicate 1 meq (or more) per 100 ml; lower case letters indicate less than 1 meq/100 ml. Abbreviation for products: a, acetic acid; f, formic acid; p, propionic acid; ib, isobutyric acid; b, butyric acid; iv, isovaleric; l, lactic acid; and s, succinic acid.

[e] Esculin hydrolysis.

[f] Starch hydrolysis.

Table 4. (Continued)

Bile growth	Catalase	Gas prod.	Indole	Meat dig.	Milk	Motility	Nitrate red	GLC analysis	Identity
*	-	1	+	-	-	-	-	Baps	Fusobacterium gonidiaformans
2	-	1	-	-	*	-	-	ASpl	Bacteroides oralis
*	-	3	+	-	*	-	+	ABps	Peptococcus indolicus
*	-	4	+	-	d	-	-	Baps	Fusobacterium necrophorum
*	-	2	-	-	a	-	-	AFLs	Eubacterium aerofaciens

[g]AMC, acetylmethylcarbinol.

[h]Gas production.

[i]Meat digestion.

[j]Nitrate reduction.

[k]Culture was lost before biochemical and physiological tests could be performed.

Species of Anaerobic Bacteria Isolated From Bovine Lung Abscesses

Table 5 lists the species of anaerobic bacteria isolated from bovine lung abscesses.

Fusobacterium necrophorum, the most common isolate, was found in 7 of the 12 culturally positive abscesses. This organism was isolated in pure culture from 3 of these abscesses (Table 4). In the past, F. necrophorum was considered a secondary invader; however, it is now known that this organism by itself is capable of causing disease (45). The possession of a cell wall lipopolysaccaride endotoxin and various types of exotoxins such as hemolysin and leucocidin probably account for the pathogenicity of this organism.

Eubacterium aerofaciens and Peptococcus indolicus together accounted for 32% of the 19 anaerobic bacterial isolates. Even though the pathogenicity of both organisms has not yet been firmly established, a report by Sorensen (67) states that some strains of P. indolicus are beta-hemolytic.

Of the 19 anaerobic bacterial isolates, Bacteroides spp. represented 11%. Bacteroides sp. (11)-2 was unique in that it was culturally inactive except for a small amount of gas production and 4+ growth in bile medium (Table 4). This type of organism is often referred to as a bile-stimulated bacteroides. In a study by Prescott (56), bile-stimulated bacteroides were frequently isolated from nonspecific infections in cattle.

Fusobacterium gonidiaformas, Bacteroides oralis and Propionibacterium acnes represented 11%, 5% and 5% of the 19 anaerobic

Table 5. Species of anaerobic bacteria isolated from bovine lung abscesses[a]

Species	No. of isolates	% of total
Fusobacterium necrophorum	7	37
Eubacterium aerofaciens	3	16
Peptococcus indolicus	3	16
Bacteroides spp.	2	11
Fusobacterium gonidiaformans	2	11
Bacteroides oralis	1	5
Propionibacterium acnes	1	5

[a]The total number of isolates was 19 (Table 4).

Table 6. Anaerobic genera isolated (19 isolates)

Genera	Percentage
Fusobacterium	47
Bacteroides	16
Eubacterium	16
Peptococcus	16
Propionibacterium	5

bacterial isolates, respectively. Although these organisms are considered pathogenic for man, only recently have they been isolated from various animal infections (5, 38, 56). The pathogenic role these organisms play in animal infections is, however, questionable at this time.

Table 6 lists the genera of anaerobic bacteria isolated from bovine lung abscesses. In this study, members of the genus Fusobacterium represented the most frequently isolated anaerobic bacteria. This completely disagrees with a report by Berkhoff (7) which states that, in animal infections, members of the genus Clostridium are the most frequently isolated anaerobic bacteria. The discrepancy may be due to the difference in number of samples examined, types of organs cultured and animal species represented (38).

Antibiotic Susceptibility and Beta-Lactamase Activity in Anaerobic Bacteria

Results of the antibiotic susceptibility tests and beta-lactamase tests are show in Table 7.

Chloramphenicol and clindamycin at concentrations of 12 µg/ml and 1.6 µg/ml, respectively, inhibited the growth of all 17 anaerobic bacterial isolates tested. In humans, chloramphenicol and clindamycin are used quite frequently in the treatment of anaerobic pleuro-pulmonary infections. However, the toxicity of these antibiotics has restricted their widespread use in treating anaerobic infections (3, 84). Clindamycin can cause the development of pseudomembranous

colitis, and chloramphenicol can act as a bone marrow depressant. The toxicity of chloramphenicol and clindamycin in cattle is questionable.

The tetracyclines, doxycycline and tetracycline, were both tested at a concentration of 6 μg/ml. Peptococcus indolicus (22)-4 and Fusobacterium necrophorum (27)-1 were the only isolates showing resistance to both doxycycline and tetracycline. Bacteroides oralis (44)-2 was resistant to tetracycline but susceptible to doxycycline. In the past, tetracycline was the antibiotic most commonly used in the treatment of anaerobic infections. Recently, however, the number of anaerobic bacteria resistant to tetracycline has drastically increased (73). Because of this, doxycycline is now considered more active against anaerobic bacteria than tetracycline, as evidenced by the results of this study.

The macrolide, erythromycin, was relatively inactive against the majority of anaerobic bacterial isolates. Only 5 of the 17 anaerobic bacterial isolates were inhibited by this antibiotic. The reason for this poor activity is that erythromycin is less active at the lower pH produced by the CO_2 atmosphere introduced during inoculation (60, 82). In future studies, oxygen-free N_2 should be used when determining the minimal inhibitory concentration of erythromycin.

Vancomycin, at a concentration of 6 μg/ml, was inactive against all isolates of Fusobacterium necrophorum and F. gonidiaformans. This supports the fact that vancomycin is often added to blood agar plates for the selective isolation of

Bacteroides spp. and Fusobacterium spp. (76). All Eubacterium aerofaciens and Peptococcus indolicus isolates were susceptible to vancomycin. Similar results were reported for these anaerobes by Kwok et al. (43).

The concentrations of the 4 beta-lactam antibiotics used in this study were: penicillin-G (2 units/ml), ampicillin (4 µg/ml), carbenicillin (100 µg/ml) and cephalothin (6 µg/ml). Fourteen of the 17 anaerobic bacterial isolates tested were susceptible to all 4 beta-lactam antibiotics. Eubacterium aerofaciens isolates were susceptible to ampicillin, carbenicillin and cephalothin, however, susceptibility to penicillin-G was questionable.

The resistance of anaerobic bacteria to beta-lactam antibiotics is dependent upon a combination of factors: 1) the affinity of the beta-lactam antibiotics for the peptidoglycan synthesizing enzymes, 2) the extent of the permeability barrier to beta-lactam antibiotics and 3) the activity of beta-lactamase present inside the bacterial cell (46). The chromogenic cephalosporin, Nitrocefin, was used in this study to compare beta-lactam antibiotic resistance with beta-lactamase activity in anaerobic bacteria. This cephalosporin was reported by O'Callaghan et al. (54) to be very sensitive to hydrolysis by a wide variety of beta-lactamases. Bacteroides asaccharolyticus SLCH and all anaerobic bacterial isolates possessed neither resistance to beta-lactam antibiotics nor beta-lactamase activity. Even though Eubacterium aerofaciens isolates showed questionable susceptibility to penicillin-G, none possessed

beta-lactamase activity. B. vulgatus VPI-8811 was resistant to penicillin-G; however, beta-lactamase activity was not detected. The beta-lactamase enzyme produced by this organism could have been a penicillinase which was more active against penicillin-G than the chromogenic cephalosporin. B. fragilis SLCH was resistant to all beta-lactam antibiotics and demonstrated beta-lactamase activity. From these results, the presence or absence of beta-lactamase activity in anaerobic bacteria, in most cases, clearly paralleled their resistance or susceptibility to beta-lactam antibiotics. In the case of B. vulgatus VPI-8811, additional mechanisms contributing to resistance must be involved.

Use of Phenethyl Alcohol in PRAS BHIA-S Roll Tubes for Isolation of Obligately Anaerobic Bacteria

As a selective agent, phenethyl alcohol has many fine qualities: it is inexpensive, virtually colorless in solution, non-toxic and can be sterilized along with the culture medium (unlike certain antibiotics). However, the most important quality is that phenethyl alcohol has been proven effective as an inhibitor of gram-negative facultatively anaerobic bacteria.

The purpose of this experiment was 1) to observe the growth of facultatively and obligately anaerobic bacteria in BHIA-S roll tubes supplemented with 6 different concentrations of phenethyl alcohol (PEA) and 2) to determine the optimum concentration of PEA required in a BHIA-S roll tube for the selective isolation of obligately anaerobic bacteria. This PEA roll tube could prove useful

for the isolation of Bacteroides spp. and Fusobacterium spp. from polymicrobial infections containing Proteus spp., Escherichia coli and other gram-negative facultatively anaerobic bacteria which tend to overgrow obligately anaerobic bacteria on noninhibitory media.

Previous investigations have shown that PEA at a concentration of 0.25% is sufficient to completely inhibit the growth of gram-negative facultatively anaerobic bacteria (8, 47). Phenethyl alcohol is considered a bacteriostatic agent toward gram-negative bacteria by its ability to inhibit selectively and reversibly bacterial deoxyribonucleic acid (DNA) synthesis. In this study, 0.25% PEA completely inhibited the growth of only 6 of the 11 gram-negative facultatively anaerobic bacteria tested (Table 8). After 72 h of incubation, Escherichia coli, Proteus mirabilis, P. morganii and P. vulgarius all showed moderate growth in roll tubes containing 0.25% and 0.30% PEA. The PEA tolerance exhibited by these 4 organisms was not anticipated and can only be explained as a variation in strains. At concentrations of 0.15% and 0.20% PEA, most of the gram-negative bacteria showed some degree of inhibition after 24 h of incubation; however, in most cases, upon further incubation a lesser degree of inhibition was observed and was attributed to an increase in tolerance to PEA.

Gram-positive facultatively anaerobic bacteria are generally considered to be resistant to the effects of PEA by virtue of their low amount of cell wall lipid (8). In this study, 8 of the 12 gram-positive facultatively anaerobic bacteria grew quite well (no

Table 8. Growth of gram-negative facultatively anaerobic bacteria in PEA[a] roll tubes

Organism[b]	Incubation[c] time (h)	Concentrations of phenethyl alcohol (%)[d]						
		0.00	0.05	0.10	0.15	0.20	0.25	0.30
Citrobacter sp.	24	++++[e]	++++	++++	++	-	-	-
	48	++++	++++	++++	+++	+	-	-
	72	++++	++++	++++	+++	+	-	-
	96	++++	++++	++++	+++	+	-	-
Edwardsiella tarda	24	++++	++++	++++	++	-	-	-
	48	++++	++++	++++	+++	+	-	-
	72	++++	++++	++++	++++	++	+	-
	96	++++	++++	++++	++++	++++	+	-
Escherichia coli	24	++++	++++	++++	++++	+++	+	-
	48	++++	++++	++++	++++	++++	++	+
	72	++++	++++	++++	++++	++++	++	+
	96	++++	++++	++++	++++	++++	+++	++
Enterobacter aerogenes	24	++++	+++	+	-	-	-	-
	48	++++	++++	++	+	-	-	-
	72	++++	++++	++	+	-	-	-
	96	++++	++++	++	+	-	-	-
Klebsiella pneumonia	24	++++	++++	++++	++++	-	-	-
	48	++++	++++	++++	++++	++	-	-
	72	++++	++++	++++	++++	++	-	-
	96	++++	++++	++++	++++	++	-	-
Proteus mirabilis	24	++++	++++	++++	++	++	-	-
	48	++++	++++	++++	++++	+++	+	+
	72	++++	++++	++++	++++	+++	+	+
	96	++++	++++	++++	++++	+++	+	+
P. morganii	24	++++	++++	++++	++++	++	-	-
	48	++++	++++	++++	++++	++++	++	++
	72	++++	++++	++++	++++	++++	+++	+++
	96	++++	++++	++++	++++	++++	+++	+++
P. vulgarius	24	++++	++++	++++	++++	++	-	-
	48	++++	++++	++++	++++	++++	++	++
	72	++++	++++	++++	++++	++++	+++	+++
	96	++++	++++	++++	++++	++++	+++	+++

Table 8. (Continued)

Organism	Incubation time (h)	Concentrations of phenethyl alcohol (%)						
		0.00	0.05	0.10	0.15	0.20	0.25	0.30
Salmonella arizonae	24	++++	++++	++++	++++	-	-	-
	48	++++	++++	++++	++++	+	-	-
	72	++++	++++	++++	++++	+	-	-
	96	++++	++++	++++	++++	+	-	-
Serratia marcescens	24	++++	++++	++++	+	-	-	-
	48	++++	++++	++++	+	-	-	-
	72	++++	++++	++++	+	-	-	-
	96	++++	++++	++++	+	-	-	-
Shigella dysenteriae	24	++++	++++	++++	++	+	-	-
	48	++++	++++	++++	++++	++	-	-
	72	++++	++++	++++	++++	++	-	-
	96	++++	++++	++++	++++	++	-	-

[a]PEA, phenethyl alcohol.

[b]From the stock culture collection of the SDSU Microbiology Department.

[c]Incubated at 37°C.

[d]Final concentrations of phenethyl alcohol in PRAS BHIA-S roll tubes.

[e]Symbols: ++++, no inhibition; +++, slight inhibition; ++, moderate inhibition; +, extreme inhibition; and -, complete inhibition.

inhibition) in all 6 concentrations of PEA (Table 9). Corynebacterium diphtheriae, C. pyogenes and Streptococcus pyogenes all showed an intolerance to high concentrations of PEA. This would seem to indicate that PEA, under proper conditions, can inhibit DNA synthesis in certain gram-positive bacteria. The fact that C. pyogenes was extremely inhibited at a PEA concentration of 0.15% is noteworthy. This organism is usually associated with Fusobacterium necrophorum in a variety of animal diseases (45). In an animal infection with both of these organisms present, the possibility exists that a PEA concentration of 0.15% or greater would allow for the selective isolation of F. necrophorum (22).

The growth of obligately anaerobic bacteria in PEA roll tubes is shown in Table 10. Except for Clostridium tertium SLCH, P. acnes SLCH and P. acnes (26)-1, all gram-positive non-sporeforming and sporeforming bacilli after 48 h of incubation showed good growth (no inhibition) in all 6 concentrations of PEA. Since Actinomyces viscosus VPI-7596 normally requires 7 to 14 days of incubation for proper growth, the inhibition of growth shown for this organism after 96 h of incubation does not accurately represent its sensitivity to PEA and therefore should be disregarded. After 72 h of incubation, all Fusobacterium necrophorum, F. gonidiaformans, Bacteroides oralis and Peptococcus indolicus isolates were either extremely or completely inhibited at a PEA concentration of 0.25%. This contradicts a report by Dowell et al. (22) which stated that all strains of Fusobacterium, Bacteroides, Peptostreptococcus and Peptococcus grew quite well on

Table 9. Growth of gram-positive facultatively anaerobic bacteria in PEA[a] roll tubes

Organism[b]	Incubation[c] time (h)	Concentrations of phenethyl alcohol (%)[d]						
		0.00	0.05	0.10	0.15	0.20	0.25	0.30
Bacillus cereus	24	++++[e]	++++	++++	++++	+++	++	+
	48	++++	++++	++++	++++	++++	+++	+
	72	++++	++++	++++	++++	++++	++++	+
	96	++++	++++	++++	++++	++++	++++	++
Corynebacterium diphtheriae	24	++++	+	-	-	-	-	-
	48	++++	+	-	-	-	-	-
	72	++++	+	-	-	-	-	-
	96	++++	+	-	-	-	-	-
C. pyogenes	24	++++	++++	++++	+	+	-	-
	48	++++	++++	++++	+	+	-	-
	72	++++	++++	++++	+	+	-	-
	96	++++	++++	++++	+	+	-	-
Listeria monocytogenes	24	++++	++++	++++	+++	+++	++	+
	48	++++	++++	++++	++++	++++	++++	++++
	72	++++	++++	++++	++++	++++	++++	++++
	96	++++	++++	++++	++++	++++	++++	++++
Staphylococcus aureus	24	++++	++++	++++	++++	+++	++	+
	48	++++	++++	++++	++++	++++	+++	+++
	72	++++	++++	++++	++++	++++	++++	++++
	96	++++	++++	++++	++++	++++	++++	++++
S. epidermidis	24	++++	++++	++	++	-	-	-
	48	++++	++++	++++	++++	++++	++++	++++
	72	++++	++++	++++	++++	++++	++++	++++
	96	++++	++++	++++	++++	++++	++++	++++
Streptococcus agalactiae	24	++++	++++	++++	+++	++	+	-
	48	++++	++++	++++	++++	++	+	-
	72	++++	++++	++++	++++	++++	+++	-
	96	++++	++++	++++	++++	++++	+++	-
S. bovis	24	++++	++++	++++	++++	++++	++++	++++
	48	++++	++++	++++	++++	++++	++++	++++
	72	++++	++++	++++	++++	++++	++++	++++
	96	++++	++++	++++	++++	++++	++++	++++

Table 9. (Continued)

Organism	Incubation time (h)	Concentrations of phenethyl alcohol (%)						
		0.00	0.05	0.10	0.15	0.20	0.25	0.30
S. faecalis	24	++++	++++	++++	++++	++++	++++	++++
	48	++++	++++	++++	++++	++++	++++	++++
	72	++++	++++	++++	++++	++++	++++	++++
	96	++++	++++	++++	++++	++++	++++	++++
S. fecium	24	++++	++++	++++	++++	++++	++++	++++
	48	++++	++++	++++	++++	++++	++++	++++
	72	++++	++++	++++	++++	++++	++++	++++
	96	++++	++++	++++	++++	++++	++++	++++
S. pyogenes	24	++++	++++	++	++	-	-	-
	48	++++	++++	+++	+++	-	-	-
	72	++++	++++	++++	++++	-	-	-
	96	++++	++++	++++	++++	-	-	-
S. uberis	24	++++	++++	++++	++++	++++	++++	++++
	48	++++	++++	++++	++++	++++	++++	++++
	72	++++	++++	++++	++++	++++	++++	++++
	96	++++	++++	++++	++++	++++	++++	++++

[a]PEA, phenethyl alcohol.

[b]From the stock culture collection of the SDSU Microbiology Department.

[c]Incubated at 37°C.

[d]Final concentrations of phenethyl alcohol in PRAS BHIA-S roll tubes.

[e]Symbols: ++++, no inhibition; +++, slight inhibition; ++, moderate inhibition; +, extreme inhibition; and -, complete inhibition.

Table 10. Growth of obligately anaerobic bacteria in PEA[a] roll tubes

Organism[b]	Incubation[c] time (h)	Concentrations of phenethyl alcohol (%)[d]						
		0.00	0.05	0.10	0.15	0.20	0.25	0.30
(11)-5	24	++++[e]	++++	++++	++++	++++	++++	++++
Eubacterium	48	++++	++++	++++	++++	++++	++++	++++
aerofaciens	72	++++	++++	++++	++++	++++	++++	++++
	96	++++	++++	++++	++++	++++	++++	++++
(15)-1	24	++++	++++	++++	++	+	+	-
Fusobacterium	48	++++	++++	++++	++	+	+	-
necrophorum	72	++++	++++	++++	+++	+	+	-
	96	++++	++++	++++	++++	+	+	+
(19)-1	24	++++	++++	++++	++++	-	-	-
F. necrophorum	48	++++	++++	++++	++++	++	-	-
	72	++++	++++	++++	++++	+++	-	-
	96	++++	++++	++++	++++	++++	+	-
(22)-2	24	++++	++++	++++	++++	++++	+	+
F. gonidiaformans	48	++++	++++	++++	++++	++++	++	++
	72	++++	++++	++++	++++	++++	++	++
	96	++++	++++	++++	++++	++++	++++	++++
(22)-4	24	++++	++++	++++	++++	++	+	+
Peptococcus	48	++++	++++	++++	++++	++	+	+
indolicus	72	++++	++++	++++	++++	++	+	+
	96	++++	++++	++++	++++	+++	++	+
(22)-6	24	++++	++++	++++	++++	+	-	-
Fusobacterium	48	++++	++++	++++	++++	+	-	-
necrophorum	72	++++	++++	++++	++++	+	-	-
	96	++++	++++	++++	++++	+	-	-
(26)-1	24	++++	++++	++++	++	-	-	-
Propionibacterium	48	++++	++++	++++	++	-	-	-
acnes	72	++++	++++	++++	+++	++	++	+
	96	++++	++++	++++	++++	+++	++	++
(26)-3	24	++++	++++	++++	++++	++	-	-
Fusobacterium	48	++++	++++	++++	++++	++	-	-
necrophorum	72	++++	++++	++++	++++	+++	-	-
	96	++++	++++	++++	++++	++++	+	-

Table 10. (Continued)

Organism	Incubation time (h)	Concentrations of phenethyl alcohol (%)						
		0.00	0.05	0.10	0.15	0.20	0.25	0.30
(27)-1	24	++++	++++	++++	++	+	-	-
F. necrophorum	48	++++	++++	++++	++	+	-	-
	72	++++	++++	++++	++++	+	-	-
	96	++++	++++	++++	++++	+	+	-
(39)-3	24	++++	++++	++++	++++	++++	++++	++++
Eubacterium	48	++++	++++	++++	++++	++++	++++	++++
aerofaciens	72	++++	++++	++++	++++	++++	++++	++++
	96	++++	++++	++++	++++	++++	++++	++++
(39)-4	24	++++	++++	+++	++	+	-	-
Peptococcus	48	++++	++++	++++	+++	++	-	-
indolicus	72	++++	++++	++++	++++	++	-	-
	96	++++	++++	++++	++++	++	+	-
(42)-1	24	++++	++++	++++	++++	+	-	-
Fusobacterium	48	++++	++++	++++	++++	++	+	+
necrophorum	72	++++	++++	++++	++++	++	+	+
	96	++++	++++	++++	++++	+++	+	+
(44)-1	24	++++	++++	++++	++++	++	-	-
F. gonidiaformans	48	++++	++++	++++	++++	++++	+	+
	72	++++	++++	++++	++++	++++	++	+
	96	++++	++++	++++	++++	++++	+++	+
(44)-2	24	++++	++++	++++	++++	++++	+	-
Bacteroides	48	++++	++++	++++	++++	++++	+	-
oralis	72	++++	++++	++++	++++	++++	+	-
	96	++++	++++	++++	++++	++++	++	+
(44)-3	24	++++	++++	++++	+++	+	-	-
Peptococcus	48	++++	++++	++++	++++	++	+	+
indolicus	72	++++	++++	++++	++++	+++	+	+
	96	++++	++++	++++	++++	++++	++	+
(44)-5	24	++++	++++	++++	++	+	-	-
Fusobacterium	48	++++	++++	++++	++	+	-	-
necrophorum	72	++++	++++	++++	++++	++	-	-
	96	++++	++++	++++	++++	++	-	-

Table 10. (Continued)

Organism	Incubation time (h)	Concentrations of phenethyl alcohol (%)						
		0.00	0.05	0.10	0.15	0.20	0.25	0.30
(44)-6 Eubacterium aerofaciens	24	++++	++++	++++	++++	++++	++++	++++
	48	++++	++++	++++	++++	++++	++++	++++
	72	++++	++++	++++	++++	++++	++++	++++
	96	++++	++++	++++	++++	++++	++++	++++
Actinomyces viscosus VPI-7596	24	-	-	-	-	-	-	-
	48	+	+	+	+	-	-	-
	72	++	++	+	+	-	-	-
	96	++	++	+	+	-	-	-
Bacteroides asaccharolyticus SLCH	24	++++	++++	++++	+	-	-	-
	48	++++	++++	++++	+	+	-	-
	72	++++	++++	++++	+	+	-	-
	96	++++	++++	++++	+	+	-	-
B. fragilis SLCH	24	++++	++++	++++	++++	++++	+++	++
	48	++++	++++	++++	++++	++++	++++	++++
	72	++++	++++	++++	++++	++++	++++	++++
	96	++++	++++	++++	++++	++++	++++	++++
B. vulgatus VPI-8811	24	++++	++++	++++	++++	++	+	+
	48	++++	++++	++++	++++	++++	++++	++
	72	++++	++++	++++	++++	++++	++++	++
	96	++++	++++	++++	++++	++++	++++	++
Bifidobacterium adolescentis VPI-C489	24	++++	++++	++++	++++	++++	++++	++++
	48	++++	++++	++++	++++	++++	++++	++++
	72	++++	++++	++++	++++	++++	++++	++++
	96	++++	++++	++++	++++	++++	++++	++++
B. eriksonii VPI-1934	24	++++	++++	++++	++++	++++	++++	++++
	48	++++	++++	++++	++++	++++	++++	++++
	72	++++	++++	++++	++++	++++	++++	++++
	96	++++	++++	++++	++++	++++	++++	++++
Clostridium perfringens SLCH	24	++++	++++	++++	++++	++++	++++	++++
	48	++++	++++	++++	++++	++++	++++	++++
	72	++++	++++	++++	++++	++++	++++	++++
	96	++++	++++	++++	++++	++++	++++	++++

Table 10. (Continued)

Organism	Incubation time (h)	Concentrations of phenethyl alcohol (%)						
		0.00	0.05	0.10	0.15	0.20	0.25	0.30
C. sordelli VPI-5917	24	++++	++++	++++	++++	++	++	+
	48	++++	++++	++++	++++	++++	++++	++++
	72	++++	++++	++++	++++	++++	++++	++++
	96	++++	++++	++++	++++	++++	++++	++++
C. sporogenes SLCH	24	++++	++++	++++	++++	++++	++	+
	48	++++	++++	++++	++++	++++	++++	+++
	72	++++	++++	++++	++++	++++	++++	++++
	96	++++	++++	++++	++++	++++	++++	++++
C. tertium SLCH	24	++++	++++	++++	++++	++++	++++	+
	48	++++	++++	++++	++++	++++	++++	++
	72	++++	++++	++++	++++	++++	++++	++
	96	++++	++++	++++	++++	++++	++++	++
Fusobacterium nucleatum VPI-8025C	24	+	+	+	+	-	-	-
	48	++++	++++	+++	++	+	+	+
	72	++++	++++	++++	++	+	+	+
	96	++++	++++	++++	+++	+	+	+
Peptococcus magnus SLCH	24	++++	++++	++++	++++	++++	++++	++++
	48	++++	++++	++++	++++	++++	++++	++++
	72	++++	++++	++++	++++	++++	++++	++++
	96	++++	++++	++++	++++	++++	++++	++++
Peptostreptococcus anaerobius VPI-5750	24	++++	++++	++++	++++	++++	+	-
	48	++++	++++	++++	++++	++++	++	-
	72	++++	++++	++++	++++	++++	++	-
	96	++++	++++	++++	++++	++++	++	-
Propionibacterium acnes SLCH	24	++++	++++	++++	++++	++++	++++	-
	48	++++	++++	++++	++++	++++	++++	+
	72	++++	++++	++++	++++	++++	++++	++
	96	++++	++++	++++	++++	++++	++++	++
Veillonella parvula VPI-8588	24	++++	++++	++++	+++	++	+	-
	48	++++	++++	++++	++++	++++	++	-
	72	++++	++++	++++	++++	++++	++	-
	96	++++	++++	++++	++++	++++	+++	-

Table 10. (Continued)

[a]PEA, phenethyl alcohol.

[b]Included anaerobic bacterial isolates (Table 4) and obligately anaerobic bacteria from the stock culture collection of the SDSU Microbiology Department.

[c]Incubated at 37°C.

[d]Final concentrations of phenethyl alcohol in PRAS BHIA-S roll tubes.

[e]Symbols: ++++, no inhibition; +++, slight inhibition; ++, moderate inhibition; +, extreme inhibition; and -, complete inhibition.

0.25% PEA agar containing 5% human blood. A plausible explanation for these inconsistent results may be the different sources of the strains. The strains tested by Dowell et al. (22) were from human infections while in this study strains represented anaerobic bacteria isolated from bovine lung abscesses. However, this explanation gives no support to the fact that Fusobacterium nucleatum VPI-8025C, B. asaccharolyticus SLCH and Peptostreptococcus anaerobius VPI-5750, all human clinical isolates, showed only a slight amount of growth at a PEA concentration of 0.25%.

In summary, by using a PEA concentration of 0.25% or greater in BHIA-S roll tubes to inhibit the growth of gram-negative facultatively anaerobic bacteria, it is very likely that the recovery of Fusobacterium spp., Peptococcus spp. and Bacteroides spp. from polymicrobial infections will be decreased. The use of PEA in PRAS BHIA-S roll tubes for the isolation of these organisms is therefore considered questionable at this time. Both gram-positive facultatively anaerobic cocci and gram-positive obligately anaerobic bacilli appear to possess approximately the same degree of high tolerance to PEA. A high concentration of PEA (0.35-0.40%) could possibly be used in BHIA-S roll tubes for the selective isolation of these organisms.

Evaluation of the SDSU Method of Identifying Anaerobic Bacteria

The purpose of this experiment was 1) to evaluate the overall accuracy of the SDSU method for the identification of anaerobic bacteria, 2) to determine the reliability of the SDSU PRAS

carbohydrate concentrate method, and 3) to evaluate the quality of SDSU PRAS bacteriological media.

Table 11 shows the results of the biochemical tests, physiological tests and gas-liquid chromotographic analyses obtained with 20 anaerobic bacteria by using the SDSU method of identification. Of the 474 tests performed, 417 (88%) of the test results were in agreement with the results of the Anaerobe Laboratory Manual (Table 11). By the results of this comparative study, the SDSU method can be considered a fairly good method for the identification of anaerobic bacteria.

Test results of the SDSU method for Bacteroides asaccharolyticus SLCH, B. vulgatus VPI-8811, Bifidobacterium eriksonii VPI-1934, Clostridium sporogenes SLCH, C. tertium SLCH and Fusobacterium nucleatum VPI-8025C showed 100% agreement with the results of the Anaerobe Laboratory Manual (Table 11). Test results for Clostridium perfringens SLCH and Veillonella parvula VPI-8588 showed 96% and 95% agreement, respectively. Test results for Peptococcus magnus SLCH, Propionibacterium acnes SLCH and P. freudenreichi subsp. shermanii VPI-0405 showed 90% agreement. Test results for Actinomyces viscosus VPI-7596, Streptococcus constellatus VPI-3810 and Bifidobacterium adolescentis VPI-C489 showed 86%, 85% 83% agreement, respectively. Test results for Clostridium chauvoei VPI-1527, Eubacterium lentum VPI-1947B, Clostridium sordelli VPI-5917, Bacteroides fragilis SLCH and Streptococcus morbillorum VPI-5424 showed 79%, 77%, 76%, 75% and 70% agreement, respectively. Test

Table 11. Results of two methods used for the identification of twenty obligately anaerobic bacteria

Organism[a]		Biochemical and physiological tests[b]																			
		Adonitol	Amygdalin	Arabinose	Cellobiose	Erythritol	Esculin pH	Esculin hyd.[d]	Fructose	Galactose	Gelatinase	Gluconate	Glucose	Glycogen	Inositol	Lactose	Maltose	Mannitol	Mannose	Melezitose
Actinomyces viscosus VPI-7596	[j]	*	*	-	*	*	-	+	a	a	*	*	a	*	*	a	a	w	*	*
	[k]	*	*	-[w]	*	*	-	v	a	a[-]	*	*	a	*	*	a[w]	a	-	*	*
Bacteroides asaccharolyticus SLCH		*	*	-	-	*	-	-	-	*	+	*	-	*	-	-	-	-	*	*
		*	*	-	-	*	-	-	-	*	+	*	-	*	-	-	-	-	*	*
B. fragilis SLCH		*	*	-	-	*	w	+	w	*	-	*	a	*	-	a	a	w	*	*
		*	*	-	-[a]	*	v	+	a	*	-	*	a	*	-	a	a	-	*	*
B. vulgatus VPI-8811		*	*	a	-	*	-	+	a	*	w	*	a	*	-	a	a	-	*	*
		*	*	a	-	*	-	+	a	*	+[w]	*	a	*	-	a[w]	a	-	*	*
Bifidobacterium adolescentis VPI-C489		*	-	a	-	*	-	+	a	*	*	a	a	-	*	a	a	a	a	a
		*	a	a[w]	a[w]	*	a[w]	+	a	*	*	a	a	a[-]	*	a	a	-[a]	a[-]	v
B. eriksonii VPI-1934		*	a	a	a	*	a	+	a	*	*	a	a	a	*	a	a	a	a	a
		*	a	a	a	*	a	+	a	*	*	a	a	a	*	a	a	a	a	a
Clostridium chauvoei VPI-1527		*	*	*	*	*	-	-	-	*	+	*	a	*	*	-	-	-	-	*
		*	*	*	*	*	-	-	w[-]	*	+	*	a[w]	*	*	w[a]	a[w]	-	w[a]	*
C. perfringens SLCH		*	*	*	*	*	-	-	a	*	+	*	a	*	*	a	a	-	a	*
		*	*	*	*	*	-	-[+]	a	*	+	*	a	*	*	a	a	-	a	*

Table 11. (Continued)

Biochemical and physiological tests																								GLC analysis[c]
Melibiose	Raffinose	Rhamnose	Ribose	Salicin	Sorbitol	Starch pH	Starch hyd.[e]	Sucrose	Trehalose	Xylose	AMC[f]	Ammonia	Bile growth	Catalase	Gas prod.[g]	Indole	Meat dig.[h]	Milk	Motility	Nitrate red.[i]	
*	a	*	w	*	*	w	-	a	*	*	-	+	*	+	*	-	-	*	-	+	SAFI
*	a	*	-[a]	*	*	a[-]	-[+]	a	*	*	-	+[-]	*	+	*	-	-	*	-	+	AF(LS)
*	-	*	*	-	*	-	-	-	-	-	-	+	-	-	2	-	-	*	-	-	ABpibivls
*	-	*	*	-	*	-	-	-	-	-	-	+	-[2]	-	2[-]	+[-]	+[-]	*	-	-	ABpibiv(ls)
*	a	*	*	w	*	a	-	-	-	a	-	+	-	+	3	-	-	*	-	-	SAfpibiv
*	a	*	*	-	*	a	+	a	-	a	-	+	4	+	-[1]	-	-	*	-	-	SAp(fibivl)
*	a	*	*	-	*	a	+	a	-	a	-	+	4	-	1	-	-	*	-	-	SApibiv
*	a	*	*	-[a]	*	a[w]	+[-]	a	-	a	-[+]	+	S[4]	-	1[3]	-	-	*	-	-	SAp(ibivl)
*	a	-	*	a	*	-	-	a	-	a	-	+	*	-	*	-	-	c	-	*	AFLs
*	a	-[w]	*	a	*	a	+	a	a[-]	a	v	v	*	-	*	-	-	c	-	*	AFLs
*	a	-	*	a	*	a	+	a	a	a	-	+	*	-	*	-	-	c	-	*	AFLs
*	a	-[w]	*	a	*	a	+	a	a	a	v	+[-]	*	-	*	-	-	c	-	*	AFLs
-	*	*	w	*	*	-	-	w	*	-	-	+	*	-	*	-	-	c	+	+	ABfps
-[w]	*	*	a[-]	*	*	-[w]	-	w[a]	*	-[w]	-	-[+]	*	-	*	-	-	a[c]	+[-]	v	AFB(sl)
-	*	*	a	*	*	w	+	a	*	-	-	+	*	-	4	-	+	c[d]	-	+	ABfpls
v	*	*	-[a]	*	*	a[-]	+[-]	a	*	-[w]	-[+]	+	*	-	4	-	+[-]	c[d]	-	v	AB(Lfps)

Table 11. (Continued)

Organism	Biochemical and physiological tests																		
	Adonitol	Amygdalin	Arabinose	Cellobiose	Erythritol	Esculin pH	Esculin hyd.	Fructose	Galactose	Gelatinase	Gluconate	Glucose	Glycogen	Inositol	Lactose	Maltose	Mannitol	Mannose	Melezitose
C. sordelli VPI-5917	*	*	*	*	*	w	+	a	*	+	*	a	*	*	-	w	-	w	*
	*	*	*	*	*	-	-+	v	*	-	*	w[a]	*	*	-	w	-	-[w]	*
C. sporogenes SLCH	*	*	*	*	*	-	+	w	*	+	*	a	*	*	-	w	-	-	*
	*	*	*	*	*	-	+	-[w]	*	+	*	v	*	*	-	-[w]	-	-	*
C. tertium SLCH	*	*	*	*	*	-	+	a	*	-	*	a	*	*	a	a	w	a	*
	*	*	*	*	*	-[a]	+	a	*	-[w]	*	a	*	*	a	a	w[a]	a[w]	*
Eubacterium lentum VPI-1947B	*	-	*	*	-	-	+	-	*	-	*	w	_	_	_	_	_	_	*
	*	-	*	*	-	-	-	-	*	-[w]	*	-	-	-	-	-	-	-	*
Fusobacterium nucleatum VPI-8025C	*	*	*	*	*	-	-	w	*	*	*	-[w]	*	*	-	-	*	-	*
	*	*	*	*	*	-	-	w[-]	*	*	*	-[w]	*	*	-	-	*	-	*
Peptococcus magnus SLCH	*	*	*	-	*	-	-	*	*	*	*	-	*	*	-	-	*	-	*
	*	*	*	-	*	-	-	*	*	*	*	-[w]	*	*	-	-	*	-	*
Peptostreptococcus anaerobius VPI-5750	*	*	*	w	*	w	-	*	*	*	*	a	*	*	w	a	*	a	*
	*	*	*	-	*	-	-	*	*	*	*	w[a]	*	*	-	-[w]	*	-[w]	*
Propionibacterium acnes SLCH	-	*	*	*	*	-	-	-	*	-	*	-	*	*	*	-	-	*	*
	-[a]	*	*	*	*	-	-	a[-]	*	+	*	a[w]	*	*	*	-	v	*	*

Table 11. (Continued)

Biochemical and physiological tests																							GLC analysis
Melibiose	Raffinose	Rhamnose	Ribose	Salicin	Sorbitol	Starch pH	Starch hyd.	Sucrose	Trehalose	Xylose	AMC	Ammonia	Bile growth	Catalase	Gas prod.	Indole	Meat dig.	Milk	Motility	Nitrate red.	
-	*	*	w	*	*	-	-	a	*	-	-	+	*	-	3	-	+	d	-	+	AFpibbivic
-	*	*	-^{w}	*	*	-	-	-	*	-	-	+	*	-	4	+	+	d^{c}	+$^{-}$	-	Aic(Fpibbivsl)
-	*	*	-	*	*	-	-	-	*	-	-	+	*	-	4	-	+	d	+	-	ABpibivvicsl
-	*	*	-	*	*	-	-	-	*	-	-	+	*	-	4^{2}	-	+	d	+	-	ABibiv(pvivsl)
a	*	*	a	*	*	a	+	a	*	a	-	+	*	-	4	-	-	c	-	-	AFLbs
a^{w}	*	*	a^{-}	*	*	a^{w}	v	a	*	v	-	-$^{+}$	*	-	4^{3}	-	-	c	+$^{-}$	+$^{-}$	ALb(fs)
*	w	-	*	*	*	a	-	w	*	*	-	+	*	-	*	-	-	*	-	+	Lafs
*	-	-	*	*	*	-	-	-	*	*	-	+	*	-	*	-	-	*	-	+$^{-}$	(afls)
*	*	*	*	*	*	-	-	-	*	*	-	+	*	-	*	+	-	*	-	*	Bafps
*	*	*	*	*	*	-	-	-	*	*	-$^{+}$	+	*	-	*	+	-	*	-	*	Baps(FL)
*	*	*	*	*	-	-	-	-	*	-	-	+	*	-	1	-	-	*	-	+	Afs
*	*	*	*	*	-	-	-	-	*	-	-	+	*	-$^{+}$	-^{2}	-	-	*	-	-	A(fls)
*	*	*	*	*	w	w	-	a	*	w	-	+	*	-	*	-	-	*	-	+	Apibivicls
*	*	*	*	*	-	-	-	-	*	-^{w}	-	+	*	-	*	-	-	*	-	-$^{+}$	A(pibbivicls)
*	*	*	*	*	-	*	*	-	-	*	-	+	*	+	1	+	-	*	-	+	APfiv
*	*	*	*	*	-^{a}	*	*	-	-	*	-	+	*	+$^{-}$	1^{2}	+$^{-}$	-	*	-	+$^{-}$	AP(Lfivs)

Table 11. (Continued)

Organism	Biochemical and physiological tests																		
	Adonitol	Amygdalin	Arabinose	Cellobiose	Erythritol	Esculin pH	Esculin hyd.	Fructose	Galactose	Gelatinase	Gluconate	Glucose	Glycogen	Inositol	Lactose	Maltose	Mannitol	Mannose	Melezitose
P. freudenreichi subsp. shermanii VPI-0405	w	*	*	*	*	-	-	w	*	-	*	w	*	*	w	-	-	*	*
	a[W]	*	*	*	*	-	+[-]	a[W]	*	-[W]	*	a	*	*	a	w[-]	-	*	*
Streptococcus constellatus VPI-3810	*	*	*	a	*	a	+	*	*	*	*	a	*	*	a	a	*	a	*
	*	*	*	a[W]	*	-[a]	+	*	*	*	*	a	*	*	-	a	*	a	*
S. morbillorum VPI-5424	*	*	*	a	*	w	-	*	*	*	*	a	*	*	a	a	*	a	*
	*	*	*	-[W]	*	-	-	*	*	*	*	a	*	*	-	a[W]	*	a[W]	*
Veillonella parvula VPI-8588	*	*	*	-	*	-	-	*	*	*	*	w	*	*	-	-	*	-	*
	*	*	*	-	*	-	-	*	*	*	*	-	*	*	-	-	*	-	*

[a] From the stock culture collection of the SDSU Microbiology Department.

[b] Abbreviations and symbols for reactions: a, acid (pH below 5.5); w, weak reaction or pH between 5.5 and 6.0; -, negative reaction; +, positive reaction; d, digestion; S, stimulated growth; c, curd; v, variable reaction; *, not tested; and numbers (bile growth and gas production), amount estimated on a "- to 4+" scale. A superscript indicates some strains react differently.

[c] Gas-liquid chromatographic (GLC) analysis was used to detect acids and alcohols produced in peptone yeast glucose (PYG) broth cultures. Capital letters indicate 1 meq (or more) per 100 ml; lower case letters indicate less than 1 meq/100 ml. Products in parentheses are not uniformly detected. Abbreviations for products: a, acetic acid; f, formic acid; p, propionic acid; ib, isobutyric acid; b, butryic acid; iv, isovaleric; v, valeric; ic, isocaproic; c, caproic; l, lactic acid; and s, succinic acid.

[d] Esculin hydrolysis

Table 11. (Continued)

Biochemical and physiological tests																						GLC analysis
Melibiose	Raffinose	Rhamnose	Ribose	Salicin	Sorbitol	Starch pH	Starch hyd.	Sucrose	Trehalose	Xylose	AMC	Ammonia	Bile growth	Catalase	Gas prod.	Indole	Meat dig.	Milk	Motility	Nitrate red.	
*	*	*	*	*	-	*	*	-	-	*	-	+	*	+	*	-	-	*	-	-	APs
*	*	*	*	*	-[a]	*	*	-	-	*	-	+[w]	*	+	*	-	-	*	-	-	APls
*	*	*	*	*	a	a	-	a	*	a	-	+	*	-	*	-	-	*	-	-	Flas
*	*	*	*	*	-	a[-]	-	a	*	-[w]	-	+	*	-	*	-	-	*	-	-	FLas
*	*	*	*	*	a	a	-	a	*	a	-	+	*	-	*	-	-	*	-	-	Flas
*	*	*	*	*	-	-[w]	-	a[w]	*	-[w]	-	+[-]	*	-	*	-	-	*	-	-	Flas
*	*	*	*	*	-	-	-	-	*	-	-	+	*	-	*	-	-	*	-	+	ap
*	*	*	*	*	-	-	-	-	*	-	-	+[-]	*	v	*	-	-	*	-	+	ap(1)

[e] Starch hydrolysis.

[f] AMC, acetylmethylcarbinol.

[g] Gas production

[h] Meat digestion.

[i] Nitrate reduction.

[j] Results of the SDSU method.

[k] Results for the Virginia Polytechnic Institute (VPI) method were obtained from the Anaerobe Laboratory Manual (39).

results for Peptostreptococcus anaerobius VPI-5750 showed only a 60% agreement. The inconsistency of the test results for the last 6 anaerobic bacteria may be a result of strain variations.

A total of 26 different SDSU PRAS carbohydrate concentrates were used, at one time or another throughout the course of this study, to determine the fermentation reactions of 20 anaerobic bacteria. Of the 229 fermentation tests performed by the SDSU PRAS carbohydrate concentrate method, 190 (83%) of the test results were in agreement with the results of the Anaerobe Laboratory Manual (Table 12). Greater than 80% agreement was obtained with all of the carbohydrate fermentation tests, except for lactose (74%), cellobiose (70%), amygdalin (67%), ribose (67%), starch (67%) and sorbitol (57%). The reason for the poor performance of these 6 particular carbohydrates is unknown. The SDSU PRAS carbohydrate concentrate method can be considered a reliable method for the fermentation testing of anaerobic bacteria. This method could be used by a small clinical laboratory not equipped to prepare or handle the 26 different PRAS carbohydrate broths needed for proper identification of anaerobic bacteria.

Seven different SDSU PRAS media were used to determine the biochemical and physiological reactions of 20 anaerobic bacteria. Of the 167 biochemical and physiological tests performed using SDSU PRAS media, 156 (93%) of the test results were in agreement with the Anaerobe Laboratory Manual (Table 13). From these results, SDSU PRAS media can be regarded as high quality PRAS media and can be used

Table 12. Carbohydrate fermentation tests: Comparison of the results of the SDSU PRAS carbohydrate concentrate method with the VPI method[a] for the identification of twenty obligately anaerobic bacteria

Carbohydrate	No. of tests[b] in agreement with the VPI method (%)	No. of tests[b] in disagreement with the VPI method (%)
Adonitol	2 (100)	0 (0)
Amygdalin	2 (67)	1 (33)
Arabinose	6 (100)	0 (0)
Cellobiose	7 (70)	3 (30)
Erythritol	1 (100)	0 (0)
Fructose	14 (93)	1 (7)
Galactose	1 (100)	0 (0)
Gluconate	2 (100)	0 (0)
Glucose	16 (80)	4 (20)
Glycogen	3 (100)	0 (0)
Inositol	4 (100)	0 (0)
Lactose	14 (74)	5 (26)
Maltose	18 (90)	2 (10)
Mannitol	12 (86)	2 (14)
Mannose	12 (86)	2 (14)
Melezitose	2 (100)	0 (0)
Melibiose	5 (100)	0 (0)
Raffinose	6 (86)	1 (14)

Table 12. (Continued)

Carbohydrate	No. of tests in agreement with the VPI method (%)	No. of tests in disagreement with the VPI method (%)
Rhamnose	3 (100)	0 (0)
Ribose	4 (67)	2 (33)
Salicin	4 (80)	1 (20)
Sorbitol	4 (57)	3 (43)
Starch	12 (67)	6 (33)
Sucrose	16 (80)	4 (20)
Trehalose	7 (100)	0 (0)
Xylose	13 (87)	2 (13)

[a]Results for the Virginia Polytechnic Institute (VPI) method were obtained from the Anaerobe Laboratory Manual (39) and are shown in Table 11.

[b]Number of tests represented the number of different anaerobic bacteria tested with a particular carbohydrate (Table 11).

Table 13. Biochemical and physiological tests: Comparison of the results of SDSU PRAS media with the VPI method[a] for the identification of twenty obligately anaerobic bacteria

Medium (test)	No. of tests[b] in agreement with the VPI method (%)	No. of tests[b] in disagreement with the VPI method (%)
Esculin (pH)	16 (80)	4 (20)
Esculin (hydrolysis)	19 (95)	1 (5)
Gelatin (gelatinase)	9 (82)	2 (18)
Chopped meat (catalase)	20 (100)	0 (0)
PYG[c] without resazurin (AMC)[d]	20 (100)	0 (0)
Chopped meat (meat digestion)	20 (100)	0 (0)
PYG agar deep (gas production)	6 (67)	3 (33)
Tryptone yeast extract (indole)	19 (95)	1 (5)
Milk (acid production, curd, digestion)	7 (100)	0 (0)

[a]Results for the Virginia Polytechnic Institute (VPI) method were obtained from the Anaerobe Laboratory Manual (39) and are shown in Table 11.

[b]Number of tests represented the number of different anaerobic bacteria tested with a particular medium (Table 11).

[c]PYG, peptone yeast extract glucose.

[d]AMC, acetylmethylcarbinol.

with confidence in the future. Gas production in PYG agar deeps was the only physiological test showing poor agreement (67%). Inconsistencies in judging the amount of gas in the agar deep could account for this poor agreement.

Tests for ammonia production, bile growth, starch hydrolysis and nitrate reduction were also included in the process of identification. Of the 58 additional biochemical tests performed, 55 (95%) of the test results were in agreement with the results of the Anaerobe Laboratory Manual (Table 11). Surprisingly, the results of the nitrate reduction tests showed an overall agreement of 88%. Nitrate reduction tests were performed using aerobically prepared Indol-Nitrite Medium. Aerobic semi-solid media can therefore be used in certain biochemical tests for the identification of anaerobic bacteria.

Results of the GLC analyses were in complete agreement with the Anaerobe Laboratory Manual (Table 11) for 18 out of the 20 anaerobic bacteria tested. Clostridium chauvoei VPI-1527 produced a small amount of propionic acid (less than 1 meg/100 ml). Propionic acid is usually not produced by this organism. Eubacterium lentum VPI-1947B produced a large amount of lactic acid (more than 1 meg/100 ml). Lactic acid is usually not produced in large amounts by this organism.

CONCLUSIONS

1. According to the results of this study, lung abscesses occur in approximately 1% of the cattle brought to the meat packing plant for slaughtering. The number of lung abscesses detected during sampling periods did not fluctuate according to seasonal changes.

2. Twelve (80%) of the 15 abscesses collected were in the diaphragmatic lobes. Aspiration of potentially pathogenic bacteria present in the oropharyngeal secretions and the *in situ* position of bovine lungs may account for the high rate of abscess formation in the left and right diaphragmatic lobes. However, further research is needed to clarify the relationship between the aspiration of oropharyngeal secretions and the formation of lung abscesses in cattle.

3. Obligately anaerobic bacteria were present in 9 (75%) of the 12 culturally positive abscesses. This high rate of incidence demonstrates that obligately anaerobic bacteria can play a pathogenic role and should not be overlooked when treating infections in animals.

4. From the 12 culturally positive abscesses, a total of 19 obligately anaerobic bacteria were isolated and identified. Each abscess yielded an average of 2 anaerobic species. Anaerobic pleuropulmonary infections in cattle, therefore, appear to be polymicrobial in nature.

5. *Fusobacterium necrophorum*, *Eubacterium aerofaciens* and

Peptococcus indolicus were the most frequently isolated anaerobic bacteria from bovine lung abscesses. Since anaerobes are normal inhabitants of the oral cavity in cattle, aspirations of oropharyngeal secretions will result in the contamination of the lung with these organisms and may eventually result in abscess formation. Therefore, organisms such as these endogenous flora of the oral cavity in cattle, will under certain circumstances become pathogenic. Further research is needed to identify additional opportunistic anaerobic bacteria present in the oral cavity of cattle.

6. According to the results of the antibiotic susceptibility tests, the beta-lactam antibiotics (penicillin-G, ampicillin, carbenicillin and cephalothin), are the most active antibiotics in vitro against anaerobic bacteria isolated from lung abscesses. Beta-lactam antibiotics are therefore recommended for treating cattle suspected of having lung abscesses.

7. The results of the beta-lactamase test used in this study indicate that the resistance of anaerobic bacteria to beta-lactam antibiotics is influenced by the production of beta-lactamase in the organisms. In some instances, failure to detect beta-lactamase activity is not indicative of the organism's susceptibility to beta-lactam antibiotics, as in the case of Bacteroides vulgatus VPI-8811 tested in this study.

8. Phenethyl alcohol in PRAS BHIA-S roll tubes is inadequate as a selective agent for the isolation of gram-negative obligately anaerobic bacteria since the growth of gram-negative facultatively anaerobic bacteria is not consistently inhibited. However, gram-positive facultatively and obligately anaerobic bacteria are tolerant to high

concentrations of PEA. A high concentration of PEA could therefore be used in PRAS-BHIA-S roll tubes to inhibit the growth of gram-negative and selectively grow gram-positive facultatively and obligately anaerobic bacteria.

9. The favorable comparison of the SDSU method results with results of the Anaerobe Laboratory Manual (39) confirms the reliability of the SDSU method for the identification of obligately anaerobic bacteria.

10. The SDSU PRAS carbohydrate concentrate method for the fermentation testing of obligately anaerobic bacteria is largely reliable, as evidenced by the agreement with the results of the Anaerobe Laboratory Manual (39). However, certain carbohydrates do show inconsistent performance. Investigation of these exceptions could provide a basis for modifications to further increase the reliability of the SDSU PRAS carbohydrate concentrate method.

11. Biochemical test results using the SDSU PRAS media showed high agreement with the results of the Anaerobe Laboratory Manual (39), indicating these media are reliable and of high quality.

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